A LABORATORY GUIDE IN HISTOLOGY

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PREFACE

The author has felt the need of a laboratory guide in this subject which would meet his own particular requirements and that is the only excuse offered for this work

It has been the aim of the author to give the student such help as experience has shown that he needs, and to direct his study by question rather than by direct statement.

This book has been designed to supplement the text and it presupposes that the student has had lectures upon the topics, as well as his own text-book study, and it should be used in no other way.

Experience has shown that while the text-books have excellent illustrations the student does not use them as he should in his microscopic study of the tissues. With this in view this work has been illustrated by sketches and photo-micrographs. These have been made by the author from his own preparations or those of his students, unless otherwise credited. The aim has not been to represent details of structure so much as it has been to show the structures as the student finds them in his own specimens. The camera lucida was used for most of the work, and the structures have been carefully labeled to aid the student in their identification and study.

It will perhaps be said that the student has received too much help and that his drawings will be made from those in his laboratory guide rather than from the section under his microscope. While this may be true to some extent, yet the fact that he has a definite plan for the study of each section, and that he knows just how to proceed, outweighs this objection in the opinion of the author who has never known a beginner in the study of Histology to say that he has had too much light thrown upon the subject.

To a certain extent histological technique has been combined with laboratory directions for study since the student proceeds more intelligently in this way. While it may seem unnecessary to repeat the directions for staining and mounting each section, yet students are found in every class to whom such advice is always helpful.

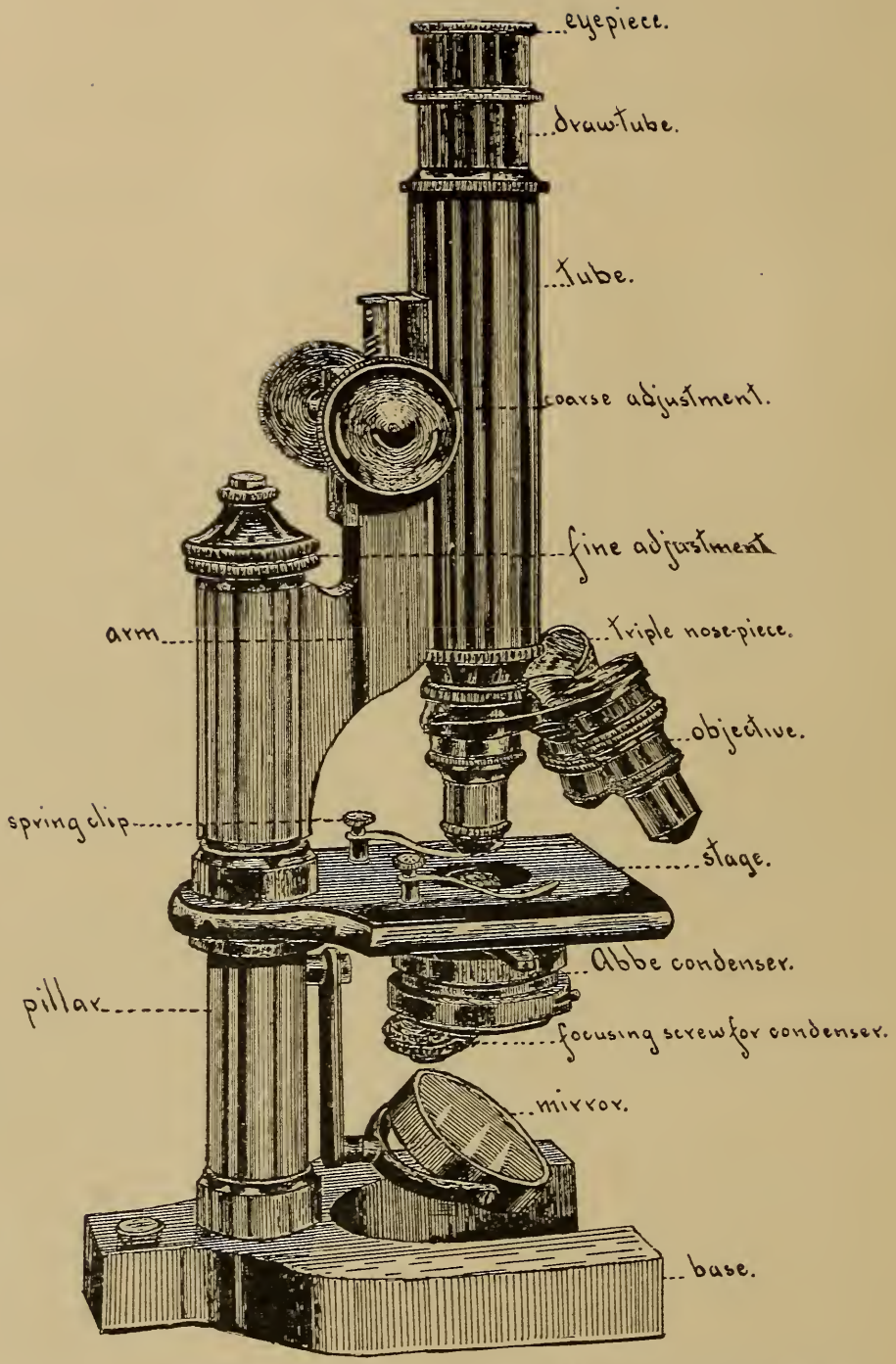
Drawings and sketches should be made even though the average medical student considers it a waste of time, for only in this way will the different structures and structural relations be fixed in mind. Sketching and drawing are very necessary adjuncts to thorough anatomical study either macroscopic or microscopic.

Only those methods in common use by students are described, detailed explanations and special technique being omitted since there are plenty of splendid works devoted to these things. The whole aim has been to give the student a simple and practical working guide. Corrections and suggestions from teachers of Histology will be greatly appreciated.

The author is indebted to his fellow-teachers in Valparaiso College for many helpful suggestions and criticisms which have aided very greatly in the preparation of this work.

CHARLES H. DEWITT.

VALPARAISO, INDIANA, November 1, 1904.



DRAWING OF A BAUSCH AND LOMB MICROSCOPE—*Yingling.*

General Suggestions to the Student.

After the student has been assigned a place at the table and a locker he will be held strictly to account for the proper care of the same.

Each student will be assigned a microscope and all of the necessary material upon the presentation of his laboratory card or breakage ticket. He will be carefully instructed in the use and care of the microscope and will be expected to use and care for the same in the proper manner. Any injury to the microscope due to carelessness upon the part of the student must be made good by the student causing such injury. If the microscope does not work properly, or if it needs any attention, report it to the instructor at once.

Reagents of any kind must not come in contact with any part of the microscope. Clean and dry the slide before placing it upon the stage of the microscope.

Filter paper, blotters, and waste material should be placed in the jars provided for that purpose. Such material must not be thrown upon the floor, table, or in the locker.

The stains, oils, and alcohols most commonly used will be furnished to the student in proper receptacles. Special stains and reagents will be secured from the instructor and should be returned to him at the close of the laboratory period. The stains and other reagents should be protected from dust and evaporation when not in use.

The drawings and sketches suggested must be made from the specimen while in the laboratory. They should be made with a hard pencil without much shading. A judicious use of colors is helpful. Drawings and their parts must be neatly labeled. The magnification should be indicated wherever possible.

Formulæ for stains and reagents will be found at the close of this work. The student is earnestly desired to familiarize himself with them so that his work may be performed with a greater degree of intelligence. The student is encouraged to ask questions bearing upon his work at all times.

General Explanation of the Process of Tissue Preparation.

(1) **Killing and Fixing.** The fresh tissue must be treated with a killing and fixing agent in order that the cells may be killed before undergoing post-mortem changes. They must now be fixed in that condition and the cell contents rendered insoluble to the reagents used in the further treatment of the tissue. The pieces of tissue should not be large so that the killing and fixing may be the more readily accomplished. The volume of the reagent should be from fifteen to fifty times that of the material.

(2) **Washing.** With the exception of the alcohols and formalin, most fixing agents must be washed out as completely as possible before proceeding further, for the reason that some fixing agents will hinder the action of the stains, if present in the tissue in excess, and in other cases precipitates form which must be removed. The washing is usually done in running water, but after certain killing and fixing fluids, alcohol must be used. If running water is not accessible a large volume of water should be used and it must be changed frequently. The best results are obtained by covering the vessel containing the tissues with mosquito netting to prevent the loss of the tissue and placing it under the tap so that the water may drip into the vessel constantly.

(3) **Hardening with Alcohol.** After washing in water the tissue will require further hardening and dehydration for which alcohol is usually used. This must be a gradual process, therefore, after washing in water place the tissue in 35% alcohol for several hours. Then use 50-60% alcohol for five or six hours, and store the tissue in 80% alcohol if not wanted for immediate use. If it is desired to imbed the tissue at once tranfer the tissue from the 80% alcohol to 95% where it is left for several hours, depending upon the size of the pieces and the character of the tissue. Transfer to absolute alcohol to complete the dehydration. This process is *very important* since a very slight amount of water in the tissue will ruin it, or at least impair its value for histological study.

(4) **Clearing.** Up to this point the process is the same whether the tissue is to be imbedded in celloidin or paraffin. Xylol or chloroform are the clearing agents commonly used prior to imbedding in paraffin and ether for imbedding in celloidin. The use of a clearing

agent at this point is to remove the alcohol and render the tissue transparent, removal of the alcohol being absolutely essential. The transfer from the absolute alcohol to the clearing oil should be made gradually.

(5) **Imbedding.** For some purposes it is merely necessary to surround the tissue with the imbedding substance, but for most work the tissues must be penetrated by and completely saturated with the imbedding medium. This latter is known as interstitial imbedding. The choice of imbedding material depends upon the use to which the tissue is to be put, and the thickness of the sections desired. The transfer from the clearing oil to the medium used for imbedding should be a gradual process. A general or *suggestive* outline for imbedding in celloidin and paraffin will be found below.

(a) **Celloidin Imbedding.**

1. Transfer from absolute alcohol to equal parts of ether and absolute alcohol for 6 to 12 hours. Why?
2. Transfer to pure ether for 6 to 12 hours. Why?
3. Place the tissue in thin celloidin for 12 to 48 hours.
4. Place the tissue in thick celloidin for 12 to 48 hours.
5. Fasten to Blocks. Select from the jar of alcohol in which they are kept a pine block having an end surface slightly larger than the piece of tissue to be blocked. Dip the end of the block into ether-alcohol and after a moment into the thick celloidin. Remove the piece of tissue from the thick celloidin, place in the desired position upon the end of the block and press it somewhat firmly against the block. Let it dry a moment and dip it into the thick celloidin or pour a little of the celloidin over the surface so as to form a coat of celloidin around the tissue. After drying a moment in the the air place the block in chloroform for a few minutes to harden the celloidin, and then complete the hardening by placing the block in 80% alcohol.
6. Cutting Sections. The sections should be cut with a sliding microtome. The tissue and knife should be flooded with 80% alcohol while cutting, and as soon as cut the sections should be transferred with a camel's-hair brush from the knife to 80% alcohol where they remain until desired for staining.

7. Staining and Mounting. Hæmatoxylin and Eosin Method.

1. Transfer the sections from the 80% alcohol to water where they are to remain for one or two minutes.

2. Stain in hæmatoxylin, 1 to 5 minutes.

3. Wash in tap-water, 1 minute. If the section is overstained place it for a few seconds in acid-alcohol and wash thoroughly in water. Instead of acid-alcohol a 2-3% solution of ammonia alum may be used. The section may remain in this solution 5 to 10 minutes after which wash in water.

4. Stain in eosin, 10 seconds to 1 minute.

5. Dehydrate in alcohol. 35%, 50%, 80%, 95%, 30 seconds to 1 minute in each up to the 95% alcohol in which it should remain for 2 or 3 minutes.

6. Clear in Eycleshymer's mixture, 10 to 20 minutes.

7. Remove the excess of the oil with a blotter, add a drop of Canada balsam, and cover with a clean, dry, cover-glass. The cover-glass should be held in the cover-glass forceps, started at one side of the section, and lowered gradually so as to drive out the air.

8. Label. The label should be numbered, the name and direction of the section given, and the name or initials of the student should be placed in the lower right hand corner.

9. General Remarks. The above is meant to be suggestive merely and it must be varied according to circumstances. The student will soon learn by his own experience as to the time required for the various operations. Should the section become cloudy after adding clearing oil it is evidence that the dehydration has not been complete and the section should be placed in 95% alcohol until completely dehydrated. If the section is cloudy after adding balsam, and it did not cloud while in the oil, it shows that the clearing was not complete, for the oil must not only render the section transparent, but it must remove the alcohol which is not a good solvent for the balsam.

(b) Paraffin Imbedding.

1. Transfer from absolute alcohol to equal parts of absolute alcohol and chloroform, 6 to 12 hours.

2. Pure chloroform, 6 to 12 hours.

3. Chloroform saturated with paraffin (cold), 5 to 6 hours.

4. Chloroform saturated with paraffin (in paraffin oven), 1 to 2 hours.

5. Soft paraffin in paraffin oven, 1 to 3 hours.

6. Hard paraffin, 50°-54°, 1 to 2 hours.

7. Imbed in paper boxes. Using paper of firm texture make small boxes by molding the paper about wooden blocks of suitable size. The instructor will show you how this is done. Number the box to correspond with the number in your tissue list. Place the tissue in the box with the surface from which it is desired to cut sections resting on the bottom and cover with the melted paraffin. Cool quickly by holding the box in ice-water, or in cold running water. After the paraffin is cold remove the paper and mark the paraffin block with a number to correspond with that of your tissue-record.

8. Cut sections.

9. Fasten the section to a *clean* slide with Mayer's fixative.

10. Heat carefully to melt the paraffin and place it in turpentine to remove the paraffin, after which treat it with xylol, 1 to 2 minutes.

11. Treat the slide with absolute alcohol, 1 to 3 minutes; 95% alcohol, 1-2 minutes; 80% alcohol, 1 minute; 50% alcohol, 1 minute; water, 1 to 3 minutes.

12. Stain in hæmatoxylin, 1 to 5 minutes. If overstained treat as for celloidin sections. The staining is more precise if overstained and then differentiated with acid-alcohol or ammonia alum. Wash thoroughly after either acid-alcohol or ammonia alum.

13. Stain in eosin, and dehydrate as for celloidin sections.

14. Clear with oil of cloves, 5 to 15 minutes, mount in balsam, and label.

The student should consult his text-book in Histology for a more detailed account of the processes of tissue preparation than can be given in this laboratory guide.

Cells.

Plant Cells. With forceps remove a small piece of the epidermis from a leaf of live-forever. Place it on a slide, add a drop of water, and cover with a cover-glass. Study with low power. Observe the following:

Fig. 1. Epidermis Live-for-ever Leaf x 200.

The large epidermal cells with their irregular outlines. Search for the nucleus, spherical in form, and more refractive than the cytoplasm. The cytoplasm is granular and grayish in color. Look for vacuoles. Sketch a few cells carefully. See Fig. 1. Using the high power, try to find cells in which there is a circulation of the protoplasm made evident by movements of the granules. Note the direction of the currents. Do you find the Brownian movement? Make a sketch and show by arrows the direction of the currents of cytoplasm.

Study of Mitosis or Karyokinesis. The root tips of the onion or of Cypripedium were fixed in a chrom-acetic mixture, hardened in alcohol, and imbedded in paraffin. Longitudinal sections were made and fastened to the slides with Mayer's albumin fixative. Take the slide given you, warm gently to soften the paraffin and place it in turpentine or xylol for several minutes until the paraffin

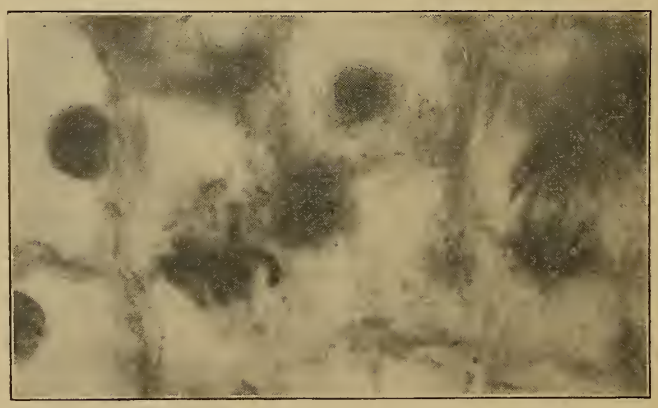


Fig. 2. Mitosis in the root-tip of Podophyllum peltatum.

is removed. Take through the grades of alcohol (absolute, 95%, 80%, etc., two or three minutes in each) to water and stain deeply with Delafield's hæmatoxylin. Remove the excess of stain with acid alcohol, wash in tap-water, stain lightly with eosin, dehydrate, clear in oil of cloves, and mount in balsam. Study under high power and trace as many of the stages of mitosis as possible. Make sketches of cells showing the various forms of mitotic figures. See Fig. 2.

Animal Cell Movements. Study under high power amœbæ obtained by allowing a bit of fish, a fresh water mussel, or material collected from a pond, to decay in water. As soon as a slight film forms on the surface a drop should be placed on a slide covered and carefully examined. Search for small and nearly colorless bodies which are slowly changing their form. How does the animal move? Can you distinguish a clearer outer zone, the ectoplasm, from the more granular endoplasm? Can you find a nucleus? A contractile vacuole? If so, study carefully and determine its function if possible. Do you find a cell-wall? Make a series of at least five sketches to show the changes in form. See Fig. 3.

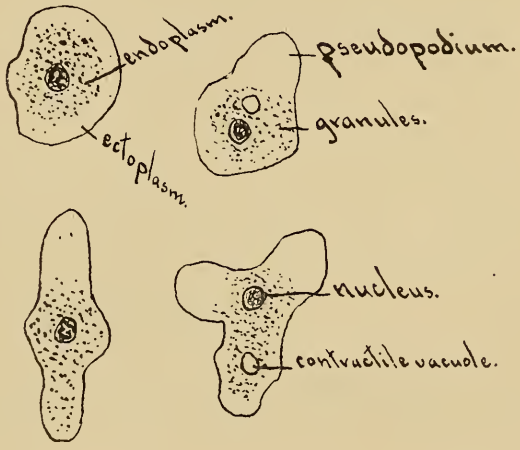


Fig. 3. Amœba. Highly magnified.

Animal Cells. Pieces of the ovary of a very young dog were fixed in bichloride of mercury or Flemming's fluid, hardened in alcohol, and imbedded in paraffin. Prepare your slide by carefully cleaning with alcohol and spread a very thin layer of Mayer's albumin fixative upon it. Avoid using too much fixative. It is well to add as little as possible and spread it with the *clean* finger tip and remove

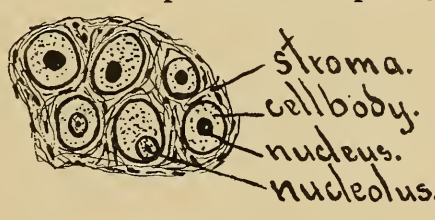


Fig. 4. Primordial Ova—Dog.

in this way as much as possible. Transfer the section from the warm water bath to the slide by placing the slide under the section and lifting it from the water and carefully blot. (Cigarette paper makes splendid blotters for this purpose). Warm carefully over a gas flame or alcohol lamp until the paraffin begins to melt and remove the paraffin with turpentine or xylol. Since dishes of xylol soon become charged with paraffin, as some one has suggested, it is a good plan to flood the slide with turpentine and drain on a blotter and then add xylol and follow with absolute alcohol, 95%, 80%, 50%, and water, if stain used be an aqueous solution, otherwise use alcohol of the same grade as that used in the stain. Stain with Delafield's hæmatoxylin and remove the excess of stain with acid alcohol, or with a 2% aqueous solution of ammonia alum, and wash in water. Stain lightly in eosin, dehydrate, clear in oil of cloves for five or ten minutes, and mount in balsam. Study under low power, noting form of cell, relatively large nuclei, nucleoli, etc. Study under high power and make sketches showing the general structure of a typical large animal cell. See Fig. 4.

Epithelial Tissues.

Squamous Epithelial Cells Unstained. Scrape the inside of the lip or cheek with a clean scalpel and mount the scrapings in a drop of physiological normal salt solution, or in the saliva, and study under high power. Note the shape and size of the cells and the shape and positions of the nuclei. Do any cells have two nuclei? Is the protoplasm clear or granular? Do you find any salivary corpuscles or leucocytes? Make sketches of several cells and label the parts. See Fig. 5.

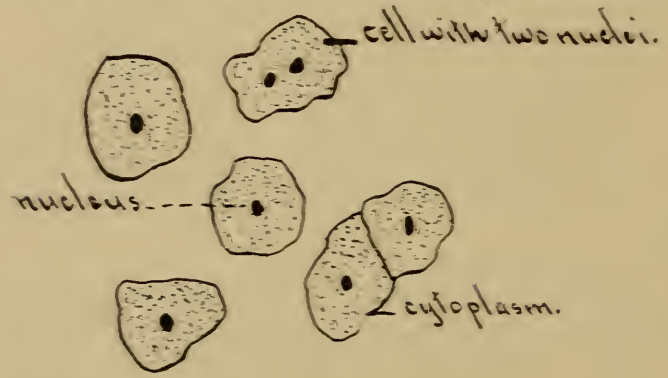


Fig. 5. Surface view Squamous Ep. Cells. Lip.

Stained Squamous Cells. Make a cover-glass preparation by spreading material obtained as above between two clean cover-

glasses and dry in the air. Fix by heat, passing quickly through the flame of a Bunsen burner or an alcohol lamp, and stain with hæmatoxylin and eosin. Dry carefully and mount in balsam. Study as above and sketch several cells.

Squamous Epithelium of a Frog. A frog was kept in a glass jar for several days until portions of the epidermis were shed. The pieces were washed in water and fixed in alcohol, after which they were stained in hæmatoxylin and eosin, dehydrated in alcohol, cleared with oil of cloves, or Eycleshymer's mixture. Take them from the oil and mount in balsam. Study the surface view of squamous epithelium thus prepared. Note the shape and size of the cells with their granular protoplasm and the intercellular cement. Do you find any intercellular spaces? Sketch a portion of the field showing form of the cells, nuclei, and their relations to each other. See Fig. 6.

Fig. 6. Epithelium Frog x 150.

Stratified Squamous Epithelium. You will receive a section of the œsophagus of a cat which was imbedded in paraffin. Fasten to a clean slide with the fixative and after removing the paraffin by gentle warming and treating with turpentine, add xylol, absolute alcohol, 95% alcohol, 70% alcohol, 50% alcohol and water.

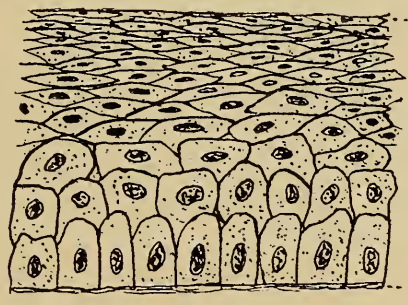


Fig. 7. Stratified Squamous Epithelium.

While it is desirable to use the grades of alcohol, it is not always done in general histological work. Stain in Delafield's hæmatoxylin, excess of which may be removed with ammonia alum (2%), or with acid alcohol, wash in tap-water, and stain lightly with eosin. Dehydrate, clear in oil of cloves, and mount in balsam.

Study the epithelial lining, first under low power and then under high power. Note the layers of cells, columnar in the lower stratum and gradually becoming flattened as they approach the surface. Do the nuclei change in shape and structure? Do you note any change in the protoplasm of the cells of the different layers? Make a sketch under high power to show the character and arrangement of the cells. See Fig. 7.

Stratified Transitional Epithelium. Pieces of bladder were fixed in alcohol, imbedded in celloidin, and sectioned. Transfer the sections to water and stain in Delafield's hæmatoxylin and eosin. Dehydrate, clear in Eycleshymer's mixture and mount in balsam. Study the epithelium under low and high power. How many strata of cells do you find? Does the number vary in different parts of the section? What is the form of the cell bodies of the deeper strata? Why do the cells vary in form? Do the superficial cells have more than one nucleus? Sketch a portion of the epithelium. See Fig. 8.

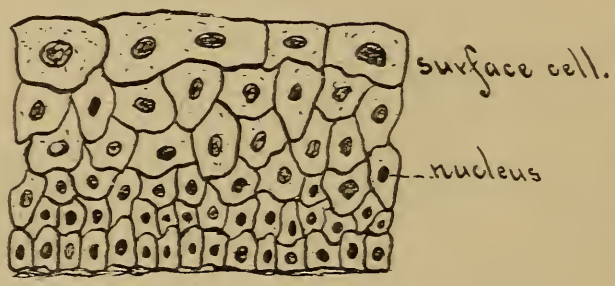


Fig. 8. Transitional Ep. Bladder.

Fresh Ciliated Epithelium. Carefully scrape the palate of a frog with a dull scalpel and mount the scrapings in normal salt solution. Study under high power noting the general form of the cell and the movement of the cilia. How do the cilia appear to move? Sketch a few cells.

Stained Ciliated Epithelium. Make a cover-glass smear of material secured as above, dry in the air and fix by passing rapidly

through a flame two or three times, or expose to the vapor of formalin for ten minutes. Stain in hæmatoxylin and eosin, wash, *dry*, and mount in balsam. Study under high power and sketch a typical cell.

Isolated Columnar Epithelial Cells. Macerate the mucous membrane of the small intestine of a cat in 33% alcohol for thirty-six hours. Tease a portion in dilute glycerin and cover. Tease another portion on a cover glass, dry and fix by passing over a flame, stain in hæmatoxylin and eosin, wash in water, dry thoroughly, and mount in balsam. Study under high power. Is the protoplasm clear or granular? Do the cells show striations? Do they possess a cuticular border? Note the shape and position of the nucleus. Sketch a few cells and label the parts.

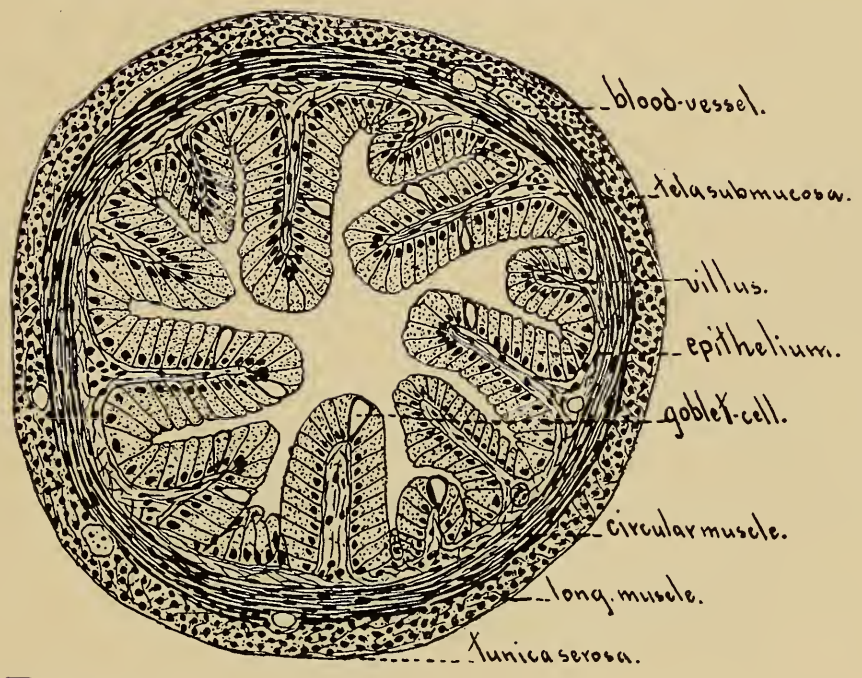


Fig. 8a. Intestine of Necturus x 30.

Columnar Epithelial Cells in Sections. The intestine of Necturus was hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin, dehydrate, clear in Eycleshymer's mixture, and mount in balsam. Study and sketch a few cells under high power. The cells of Necturus are very large and easily studied. See Fig. 8a.

Mesothelium and Endothelium.

Mesothelium. Carefully remove the mesentery from the intestine of a cat, rinse thoroughly in distilled water to remove any foreign substances that may be present, and place in the dark in a 1% solution of silver nitrate from ten to fifteen minutes, or until it becomes somewhat opaque. Rinse in distilled water and expose to the light in water or in 10% formalin until the silver is reduced giving a brown color. Rinse in water, stain in hæmatoxylin, dehydrate, clear, and mount in balsam. It is probably best to pin out the mesentery on thin sheets of cork until after the dehydration and clearing as it prevents the rolling and shriveling of the material. Study under high power, noting the shape and relation of the cells. Do you find intercellular spaces? Do you find stomata or pseudostomata? Sketch a few cells carefully. See Fig. 9.

Fig. 9. Mesothelium x100.

Endothelium. The endothelial cells of the mesenteric capillaries are best shown by injecting the vessels of a small animal through the thoracic aorta. Chloroform a cat and when completely anæsthetized, open the thorax and incise the heart so as to remove as much of the blood as possible. Inject from 75 to 100 c. c. of a 1% solution of silver nitrate, and after fifteen or twenty minutes inject from 100 to 150 c. c. of a 10% solution of formalin. After a few minutes open the abdominal cavity and expose the mesentery to the light. When the reduction of the silver has occurred, remove

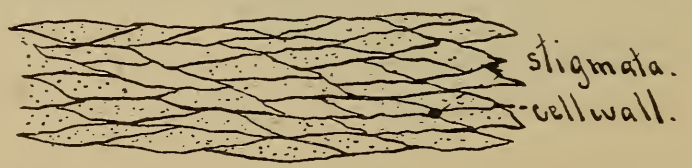


Fig. 10. Endothelium of Frog. Silver nitrate.

pieces of the mesentery, wash in water, study, and sketch under high power. See Fig. 10.

Fibrous Connective Tissue.

Unstained White Fibrous Tissue. Tease the portion of a tendon of an ox given you in salt solution, cover and study under the low power and then under the high power. How are the fibrillæ arranged? Do they branch or anastomose? Sketch. See Fig. 11. Add a little dilute acetic acid (1%) at edge of coverglass and draw it under with a piece of filter paper. What is the effect of the acid? Boil a piece of the tendon for some time. What is formed? What are the characteristics of white fibrous tissue?

Fig. 11. White Fibrous Tissue of Tendon of Ox.

Stained White Fibrous Tissue. A piece of tendon was hardened in alcohol, stained in borax-carmine, dehydrated, imbedded in paraffin, and sectioned longitudinally. Fasten to a slide, remove the paraffin with xylol, and mount in balsam. Are cells present? How are the fibers arranged? Study and sketch under high power.

Fresh Yellow Elastic Tissue. Tease a portion of the ligamentum nuchæ of the ox in salt solution, cover and examine. Describe the general appearance of the fibers. Do they branch or anastomose? What position do the free ends assume? Are the fibers cemented into bundles? Are white fibers present? Add acetic acid as above. What is the result? Boil as above. What is formed as a result of the boiling? What are the characteristics of yellow elastic tissue? Sketch. See Fig. 12.

Stained Yellow Elastic Tissue. A portion of the ligamentum nuchæ was hardened in alcohol, stained in borax-carmine, imbedded in paraffin, and sectioned longitudinally. Fasten to the slide, remove the paraffin, and mount in balsam. Study and sketch under high power.

Stained Yellow Elastic Tissue. The above was repeated for a cross section. Study under high power, noting the grouping into bundles and the angular shape of the fibers. Do you find any cells? Sketch a small portion as seen under the high power.

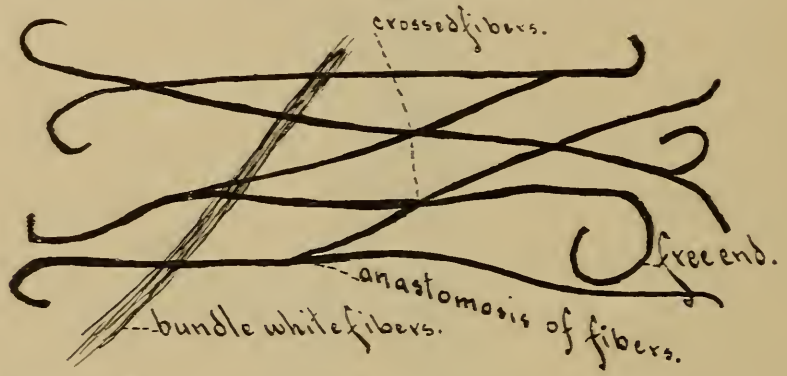


Fig. 12. Elastic Fibers from Lig. Nuchæ Ox.

Areolar Connective Tissue. With your fine forceps remove a portion of the subcutaneous tissue of a cat or rabbit and place on a dry slide and, if the ends are drawn out and the center kept moist by breathing upon it, a thin film may be obtained which should be covered with a cover-glass having a drop of salt solution upon it. The tissue may be teased in the salt solution but it is less satisfactory than the above method. Study under high power. How is the white fibrous tissue arranged? Note the arrangement of the elastic fibers. Sketch a portion of the field and label the parts. Add dilute acetic acid as above and note the effect upon the different tissues. Can you distinguish fixed connective tissue cells? Sketch a portion of the field so as to show the different tissues.

Embryonic Connective Tissue. A portion of human umbilical cord was hardened in formalin, imbedded in celloidin, and sectioned. Stain your section in hæmatoxylin and eosin, dehydrate, clear, and mount in balsam. Study under high power. Note the branching connective tissue cells. Do they anastomose?

Do you find any intercellular substance? Do you find fibers? Cross sections of blood vessels will be seen, but do not study them. Sketch a portion of the field showing form and relation of the cells.

Fat Cells. The fatty mesentery of a dog was pinned to cork, fixed in alcohol, stained in hæmatoxylin, washed in tap-water, dehydrated, and cleared in oil of bergamot. Mount in balsam. Study under low power. The fat cells are clear and round or oval. How are they arranged? What is their relation to the blood-vessels? Sketch a group of the cells. Search for nuclei, using the high power. Sketch a typical cell.

Cartilage.

Hyaline. The larnyx of a rabbit was fixed in corrosive sublimate, hardened in alcohol, and imbedded in celloidin. Transfer the sections to water and stain in hæmatoxylin and eosin, dehydrate, clear, and mount in balsam.

Fig. 13. Hyaline Cartilage from the Larynx.

Study under high power. What is the character of the matrix? What is the shape of the cells? Do they occur singly or in groups? Can you discern capsules around the cells? What is the structure of the perichondrium? Note the change in the cells as you pass from the perichondrium toward the center of the cartilage. Make a sketch. See Fig. 13.

White Fibro-cartilage. A portion of interarticular fibro-cartilage was fixed in corrosive sublimate, hardened in alcohol, and imbedded in celloidin. Stain deeply in hæmatoxylin and then in Van Gieson's stain, dehydrate rapidly, clear in oil of origanum, and mount in balsam.

Study carefully using the high power. What is the composition of the matrix? Are the cartilage cells encapsulated? Do you find any hyaline cartilage around the cells? Why is this? Sketch a portion of the section showing the structural relations.

Elastic Fibro-cartilage. The epiglottis was fixed in corrosive sublimate, hardened in alcohol, and imbedded in celloidin. Stain with picro-carmine or with the elastic tissue stain used by H. G. Harris. Stain for about ten minutes, wash one minute in 1% nitric acid in 60% alcohol, dehydrate with 95% alcohol, clear, and mount in balsam. How are the cells arranged? Are they surrounded by hyaline cartilage? How may the elastic tissue be recognized? What is the arrangement of the elastic tissue? Sketch a portion as seen under high power so as to show the form and the relation of the tissues.

Fig. 14. Long. Section of Metacarpal Bones of a Fœtus; *a*, hyaline cartilage; *b*, interosseous muscle; *c*, center of ossification.

Glycogen in Cartilage Cells. Treat *fresh* cartilage with dilute Lugol's solution, and glycogen may be found in the cartilage-cells, stained a peculiar brown color, usually described as mahogany brown. Study and sketch a few cells showing glycogen.

Calcification and Ossification of Cartilage. The hand of a small human fœtus was fixed in formalin, dehydrated, and imbedded in celloidin. Stain with hæmatoxylin and eosin, and mount in balsam. Study under low power. Note that there are well defined areas shown in a long bone.

Where is hyaline cartilage found? Do you find perichondrium or periosteum? The areas should appear as follows if taken in order from the articular surface toward the center: (a) Hyaline or articular cartilage. (b) An area in which the cartilage cells are somewhat flattened and arranged in longitudinal rows. (c) An area in which the cartilage cells are greatly enlarged, perhaps distending the capsules. (d) An area in which the cells seem shrunken and showing degeneration of the nuclei. (e) Area of ossification in which the primary marrow spaces are to be seen, as well as calcified cartilage trabeculæ around which the osteoblasts are depositing layers of spongy bone. Sketch a portion so as to show a portion of each area in its proper relation to the other areas. See Fig. 14.

Bone.

With the saw make thin transverse sections of a dry, clean metacarpal bone. Smooth one surface by rubbing on a fine file and then polish it on a fine hone. Using printer's paste or glue, fasten the sections on a smooth pine stick, allow the paste or glue to dry and then file the sections thin enough that the grain of the wood is easily seen through the section. Remove the sections by soaking in warm water, dry and pol ish on a fine hone, rubbing until an examination under low power shows the structures clearly. When perfectly dry mount on a slide in the following manner: Place a little *very thick balsam* on a slide and a little on a cover glass. Heat the slide and cover carefully and cool until a film forms, place the section on the slide, cover quickly, and press cover down firmly.

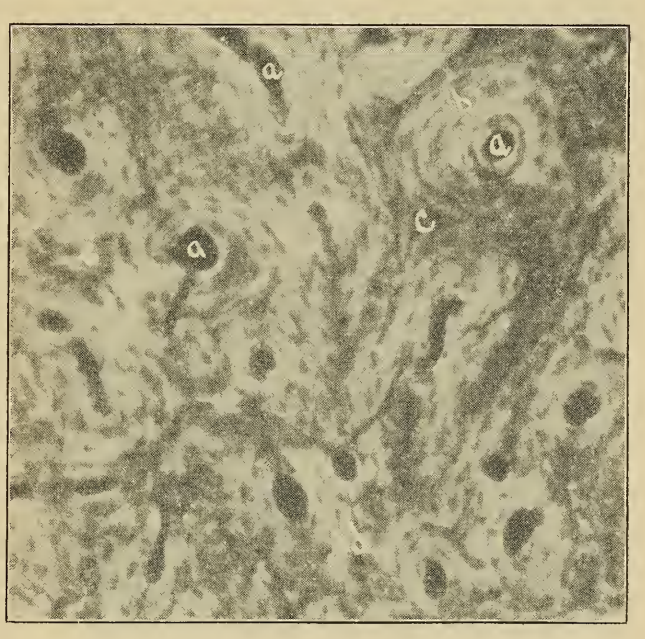


Fig. 15 Transverse Section Bone of Ox: *a*, Haversian canal; *b*, bone of Haversian system; *c*, intersystemic bone; *d*, lacunæ.

Study the transverse section first under low power and then under high power. The Haversian canals with the concentric lamellæ of bone surrounding them are easily recognized. What is the nature of the matrix? Study the outer and inner circumferential lamellæ. Do you find Volkmann's canals? Note the interstitial or intersystemic bone between the concentric Haversian systems. What is the position of the lacunæ? Do the canaliculi anastomose? Do the canaliculi of the adjacent systems anastomose? Do the Haversian canals anastomose? Sketch a portion under high power? See Fig. 15.

Teeth.

Sections of teeth may be made in the same manner as that described above for bone, if desired. The jaw of a young kitten or dog was placed in a mixture composed of 10% formalin 1 part, and 10% nitric acid 1 part, until the bone was decalcified when pieces were imbedded and sectioned transversely and longitudinally. Fair sections may be made without imbedding as the pieces may be held in the microtome clamp. Stain the sections in hæmatoxylin and eosin, and mount in balsam.

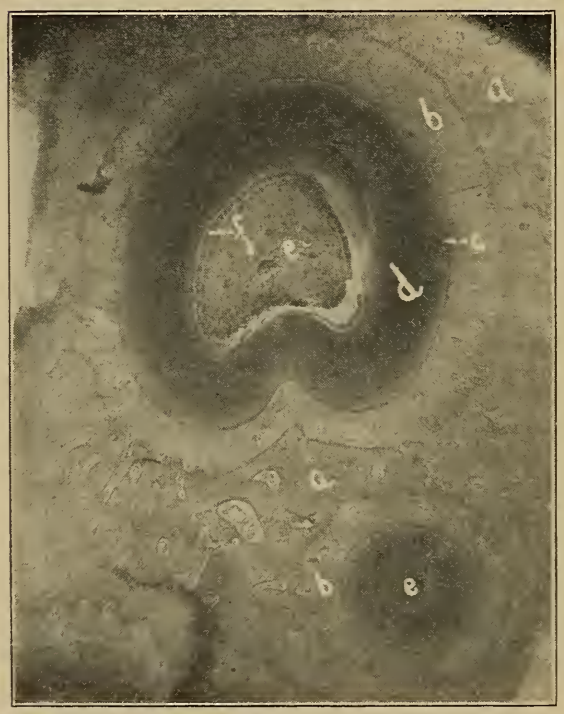


Fig. 16. Transverse Section Jaw and Teeth of a Cat: *a*, bone of a jaw; *b*, dental periosteum; *c*, cementum; *d*, dentine; *e*, pulp; *f*, odontoblasts.

Study the transverse section of tooth and jaw under low power beginning at the center. What is the nature of the pulp? Surrounding the pulp and intimately connected with it by means of processes are the odontoblasts, cells columnar in form.

Next in order is the den-

tine composed of dentinal tubules lying in the intercellular substance or matrix. Do they branch?

Next in order is the granular layer of Tomes with its interglobular spaces.

Next in order is the cementum. How does it differ in appearance from the bone you studied?

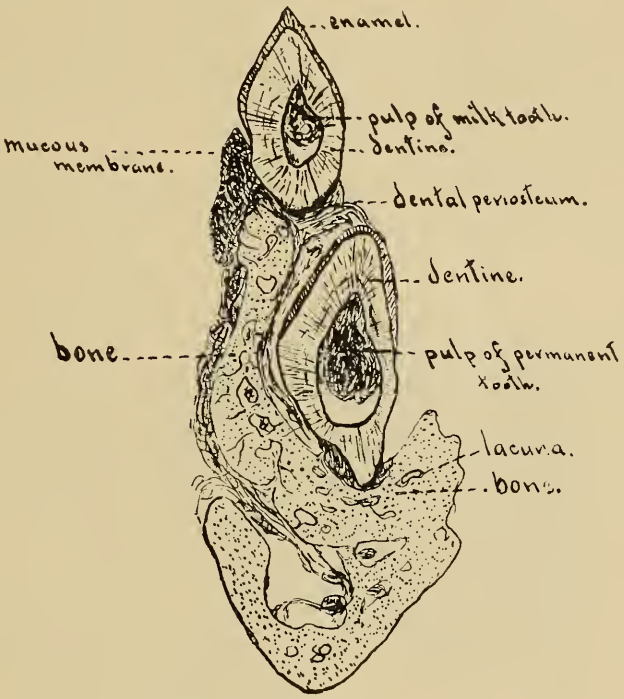


Fig. 17. L.S. Jaw and Teeth of a Kitten.

Next in order is the dental periosteum. What is its structure? Draw the section under low power. See Fig. 16.

Study your longitudinal section of tooth and jaw in the same manner. Many sections will show the permanent tooth beneath the temporary. If so, study their relations. This section is designed to show the enamel and its relations and should be carefully studied. Make a sketch under low power showing the general relations. See Fig. 17.

Muscle.

Fresh Striated or Skeletal. (a) Tease a portion of the thigh muscle of a frog in physiological normal salt solution to isolate the fibers, and study under low and high powers. Do the fibers

show striæ? Do you find the sarcolemma? Broken fibers usually show the sarcolemma. Add a little dilute (.75%) acetic acid at the edge of the cover-glass and draw it under by holding filter paper at the opposite side. Where are the nuclei located? Do you find fibrillæ? Make a sketch to show the sarcolemma in a broken fiber.

(b) Tease the muscle of Hydrophilus, which has been fixed in alcohol, in dilute glycerin and study under high power. This preparation shows the details of striation better than most preparations. Sketch a few fibres.

(c) **Longitudinal Section Human Striated Muscle.** Stain the section in hæmatoxylin and eosin and mount in balsam.

Fig. 18. T.S. Human Muscle × 100.

Study under both powers and sketch a small portion under high power. Where are the nuclei located? How are the fibers held together? What makes the longitudinal striations? Are the cells uni-nucleated or multi-nucleated?

(d) Longitudinal Section of Injected Muscle. The muscle of a cat injected with carmine-gelatin was imbedded in celloidin, after hardening in alcohol, and sectioned longitudinally. Study and sketch under low power showing the injected vessels and their relation to the fibers.

(e) Transverse Section Human Muscle. Tissue was fixed and hardened in alcohol, imbedded in celloidin, and sec-

tioned. Stain the section in hæmatoxylin and eosin and mount in balsam. Study under low and high power. What is the position of the nuclei? What are Cohnheim's areas? Study the connective tissue of the muscle and the fibers. Sketch a small portion of the field under the high power. See Fig. 18.

Cardiac Muscle. (a) Macerate cardiac muscle for twelve to twenty-four hours in fuming nitric acid of 20% strength. Wash out the acid in water and shake the muscle vigorously in a long test-tube, or tease gently on a slide, and study under high power. What is the shape of the cells? Do they branch? Are they striated? What is the form and number of nuclei? Sketch two or three cells.

(b) The heart of a cat was fixed in bichloride of mercury, hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin. Study under low and high powers. Do the bundles of fibers anastomosé? What is the position of the nuclei in cross sections?

Nonstriated Muscle. (a) Isolated Cells—Macerate and study as above for cardiac muscle. Make a sketch to show form and structure of the cells.

(b) Study the arrangement and appearance of nonstriated muscle in section by using the intestine of Necturus which has very large cells. Sketch a portion under high power showing longitudinal and transverse sections of muscle. See Fig. 8a.

Blood.

Fresh Human Blood. Obtain a small drop of blood by pricking the finger just below the root of the nail. The finger should be cleaned carefully and washed with alcohol and the needle should be sterilized in a flame. Place a drop on a clean slide and cover; mix a second drop with physiological normal salt solution and cover; place a large drop of blood on a third slide and allow clotting to commence before adding a cover-slip. Study the first preparation, noting that the red corpuscles tend to collect in rouleaux. Search for white corpuscles. Make drawings to show the rouleaux and the relative size of white

and red corpuscles. Make a drawing of a red corpuscle on edge.

Examine the second preparation in the same way, noting any differences in appearance. Do the red corpuscles collect in rouleaux? Why? Keep this second slide at 40° C., if possible, and try to get a series of sketches showing amœboid movement of the white corpuscles.

The third preparation should be washed gently to remove as many of the red corpuscles as possible. The section may now be stained with methylene blue and examined under a high power. How are the fibrin filaments arranged? Do they

Fig. 19. Human Blood highly magnified.

stain? Sketch. The nuclei of white corpuscles will be stained a deep blue.

Stained Human Blood. (1) Spread a drop of blood between two *clean* cover-glasses and quickly draw them apart and let the film dry. Place in forceps and fix by passing quickly through the flame. Stain in hæmatoxylin and eosin, dry carefully, and mount in balsam. The nuclei of the white cells stain with the hæmatoxylin while the eosin stains the protoplasm of the corpuscles and more deeply the eosinophile granules of the polynuclear cells. Examine with high power, noting the following forms of cells:

(a) Erythrocytes stained with eosin.

(b) Small lymphocytes, mononuclear, nucleus relatively large and staining deeply, small amount of protoplasm surrounding the nucleus.

(c) Larger mononuclear cells having a nucleus which stains less deeply than (b).

(d) Transitional forms with U shaped nucleus.

(e) Polynuclear cells with nuclei separate or lobulated and joined by fine threads of nuclear material. The nuclei stain quite deeply in most instances. Make drawing to show the various forms as seen under high power. See Fig. 19.

(2) Make a preparation of blood on a clean slide by removing a drop with the end of a slide. Draw the second slide along on the first, holding it at an angle of 45°, thus leaving a thin film on the first slide. You will probably get a better preparation in this way than by using cover-glasses. Place the slides in formalin vapor for ten minutes and stain in methylene blue and eosin. Wash, dry, and mount in balsam. Study as above, noting the result obtained with this method of fixation as compared with the dry method. Preparations may be made by treating the films with equal parts of ether and absolute alcohol for a number of hours, and then staining as above, or with hæmatoxylin and eosin, but, for general work, formalin vapor is greatly to be preferred.

Frog or Turtle Blood. (1) Make cover-glass preparations, fix in the dry way, and stain with hæmatoxylin and eosin. Make drawings showing the nucleated red cells and the relative size of white and red cells.

(2) Study the amœboid movement of the white cells. Ring a cover-glass with vaseline and use it to cover a drop of blood mixed with a drop of normal salt solution. Find a white cell showing amœboid movement and make six drawings, at two or three minute intervals, showing the changes in form. Can you distinguish ectoplasm and endoplasm? Are the pseudopodia clear or granular?

Bird Blood. Make and stain preparations of the blood of the English sparrow and study as above. It would be well to make drawings showing form and relative size of the erythrocytes of the various forms of blood that you have studied.

Hæmin or Teichmann's Crystals of Human Blood. Place a drop of blood on a slide, add a small crystal or two of common salt, and after drying add a drop or two of glacial acetic acid, cover and heat gently over a flame until the acid boils. Cool and irrigate with water, or allow the acid to evaporate, and when *perfectly dry* mount in balsam. Study and make drawings of the brownish-black crystals, noting carefully their form, size, and arrangement. See Fig. 20.

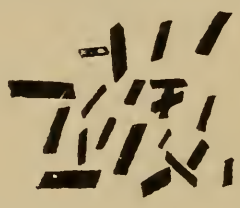


Fig. 20. Hæmin Crystals.

Hæmin Crystals of the Sparrow. Prepare as above and compare carefully with those from human blood. Show the difference and also resemblance by means of sketches.

Hæmin Crystals of Cat, Dog, and Rat. Prepare and study as above.

Hæmin Crystals From Blood-stained Cloth. The portion of the cloth which you will receive has upon it dried blood which should be prepared as follows: Tease it on a slide in normal salt solution or in distilled water, add a few crystals of salt and set aside until the stain soaks out into the solution. Now remove the fibers and let the solution evaporate. The evaporation may be hastened by *gentle* warming; when dry add glacial acetic acid and finish the preparation as above. Examine carefully and try to identify the crystals. You will remember that this test is *only to show that blood is present*, as the crystals from pigeon's blood have much the same form and size as those of a mammal. Hæmin crystals are chlorides of hæmatin, an iron compound found in the hæmoglobin.

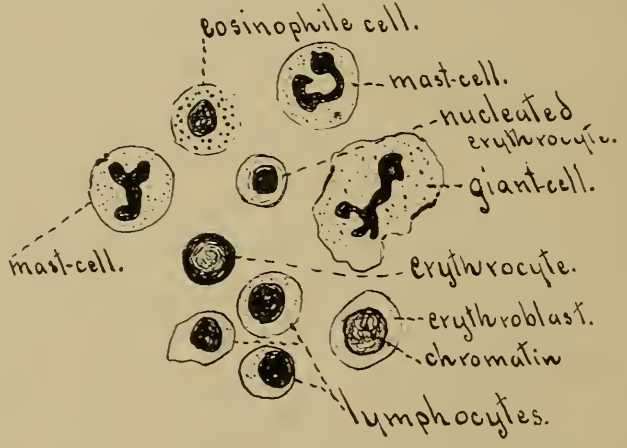
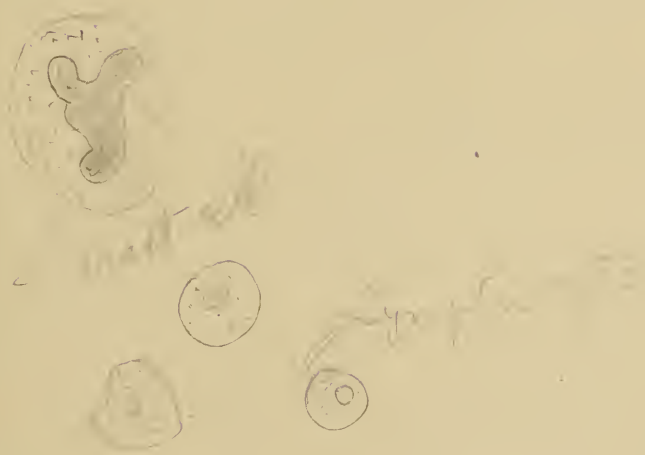


Fig. 21. Cells from Red Marrow of a Dog.

Study of a Blood-clot. Take a portion of the blood-clot given you, place it on a slide and cover with a 30% solu-



tion of potassium hydrate until the clot softens when it is to be teased and examined for corpuscles. Can you identify them? Do they belong to bird, mammal, or amphibian? Hand a written report of your findings to the instructor.

Red Marrow of Bones. From the red marrow of the bone of a cat or dog make several cover-glass preparations. Fix one by the dry method and stain in hæmatoxylin and eosin. Fix another in formalin vapor for ten minutes and stain in methylene blue and eosin. Fix one in equal parts of ether and absolute alcohol for twenty-four hours and stain in hæmatoxylin and eosin. Mount in balsam. Study under high power and make sketches of the various kinds of cells that you find. How do the marrow cells differ from leucocytes?

(a) Search for erythroblasts having large nuclei with distinct chromatin threads, sometimes showing a tinge of color in the protoplasm.

(b) Look for normoblasts having hæmoglobin with globular deeply stained nuclei and no chromatic filaments. What becomes of the nuclei as they are transformed into erythrocytes?

(c) Look for mononuclear eosinophile cells and transitional eosinophile cells.

(d) Search for polynuclear cells and lymphocytes.

(e) Try to find giant cells with polymorphous and simple nuclei. See Fig. 21.

Blood Vessels.

The aorta of a dog was fixed in formalin, hardened in alcohol, and imbedded in paraffin. Fasten a section to a slide with albumin fixative, remove the paraffin, stain in hæmatoxylin and eosin, and mount in balsam. Study under low and high power. Three coats or tunics should be found from the outer to the inner as follows:

(1) The tunica adventitia which is not very thick. What is its composition? How are the elastic fibers disposed?

(2) The tunica media or middle coat consists largely of elastic tissue in concentric layers with very few smooth muscle cells. The tunica media is separated from the tunica intima and the tunica adventitia by what are known as elastic limiting membranes. Do you find these membranes? What is their structure?

(3) The tunica intima consists of a single layer of flattened endothelium, the nuclei of which project into the lumen of the vessel, and a sub-endothelial fibrous layer richer in cells in the inner than in the outer portion next to the internal elastic limiting membrane. What kind of fibrous tissue do you find in the intima? Sketch a portion of the vessel and label the parts. See Fig. 22.

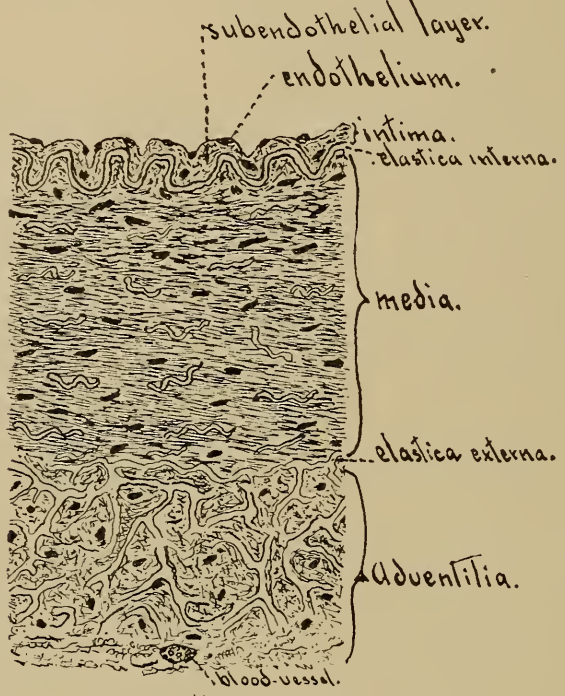


Fig. 22. T.S. Medium-sized Human Artery. x 200.

Medium Sized Vein. Stain the section in hæmatoxylin and Van Gieson's stain and mount in balsam. Study carefully under high power and sketch a section. The tunica intima consists of three layers :

(1) A single layer of endothelial cells.

(2) A layer of smooth muscle the bundles of which are somewhat separated by white fibrous connective tissue.

(3) A fibro-elastic layer containing more white fibers than that of the artery. The tunica media, separated from the intima by an internal elastic membrane, contains circularly arranged muscle cells sometimes forming a continuous layer but often broken by strands of fibrous tissue. Compare with the media of an artery of the same size. The tunica adventitia has an inner longitudinal muscular layer which is usually quite prominent. The adventitia is thicker than in the artery and has a greater amount of the white fibrous tissue. See Fig. 23.

Capillaries. Anæsthetize a cat and open the thorax. Open the left ventricle and remove as much blood as possible. Now inject a 1% solution of silver nitrate and after half an hour open the

abdominal cavity, remove the mesentery and pin it out on sheets of cork and place in 10% formalin or 95% alcohol, and expose it to the sunlight until the tissue assumes a brownish tint. Dehydrate, clear in oil of bergamot or in Eycleshymer's mixture, and mount in balsam. The nuclei may be stained with hæmatoxylin if desired. Study under high power searching for capillaries and the smaller arterioles, the endothelial cells of which should be outlined in brown or black lines. What is the shape of the cells? Is the outline regular or irregular? Do you find any intercellular spaces? Make a drawing. See Fig. 10.

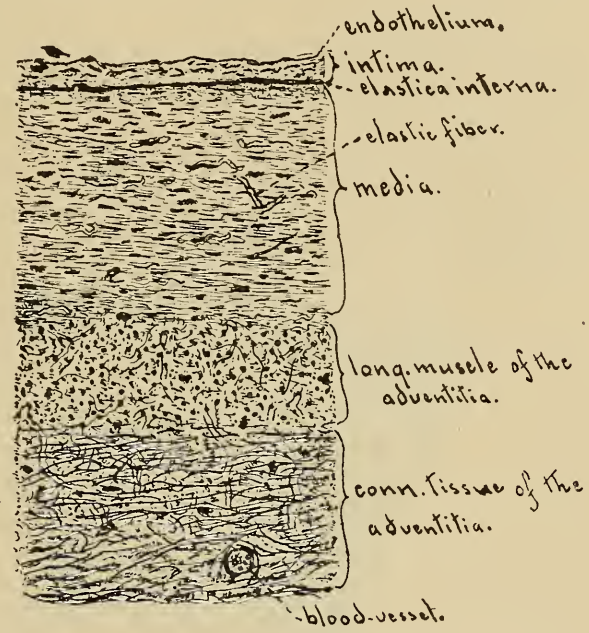


Fig. 23. T.S. Vein showing the Tunics.

Adenoid or Lymphatic Tissues.

Diffuse adenoid tissue is found in the mucosa of the intestinal and respiratory tracts as ill-defined masses of lymphatic tissue. Search for it in sections of larynx, stomach, and colon. The reticulum is usually partially or entirely obscured by the very numerous lymphoid cells. You may find it in places, however. Sketch a portion under the high

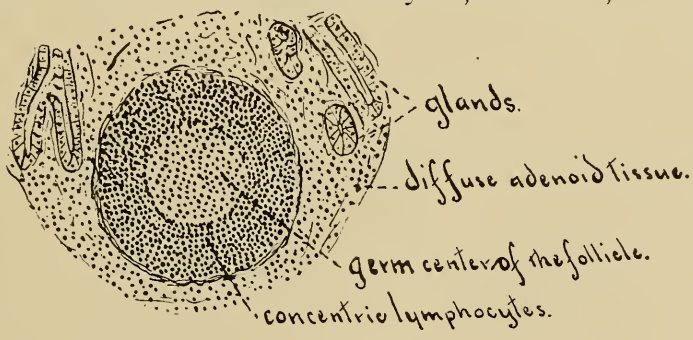


Fig. 24. Solitary Lymph-gland. Duodenum.

power. The section of the appendix of a rabbit is excellent for studying diffuse adenoid tissue, though nodules are fairly defined in the submucosa.

Solitary Gland. Using the low power, search for solitary glands or lymphoid nodules in your section of the colon of a dog. Make a drawing to show the structure and relation to the surrounding tissue. See Fig. 24.

Thymus Gland. The thymus gland of a rabbit was fixed in a saturated solution of corrosive sublimate in normal salt solution, hardened in alcohol, and imbedded in celloidin. Stain in hæma-

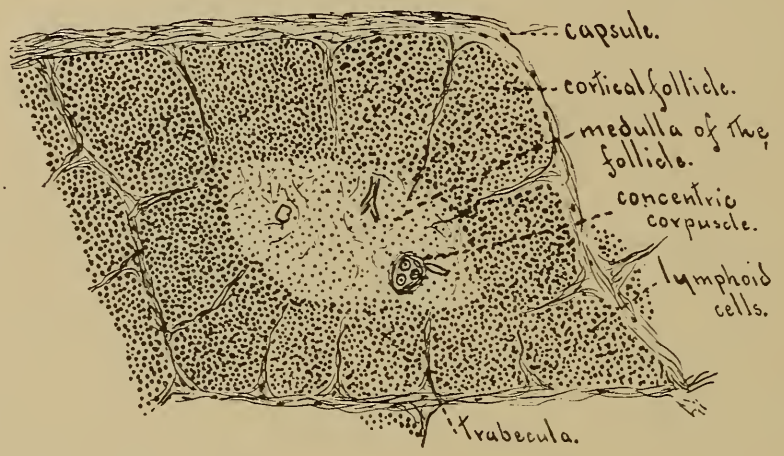


Fig. 25. Section of Human Thymus. Slightly diagrammatic.

toxylin and eosin and mount in balsam. Study first under low power noting the fibrous capsule from which septa pass dividing it into lobules. The lobules are likewise divided into secondary lobules within which are the follicles of adenoid tissue. Note the difference between the cortical and medullary zones of the follicles. Search for the corpuscles of Hassal. What is their nature? How may they be recognized under the microscope? Sketch a follicle as seen under high power. See Fig. 25.

Lymph Gland. A lymph gland was fixed and hardened in alcohol and imbedded in paraffin. Fasten a section to the slide and stain in hæmotoxylin and eosin. Some authors recommend Flemming's solution and staining with safranin, especially for the study of the germ centers of the follicles. Study under low power, noting the connective tissue capsule. What elements enter into its composition? Note that primary trabeculæ radiate toward the hilus

from the capsule, thus dividing the gland into lobes. Note the secondary trabeculæ given off from the primary. Tertiary trabeculæ are given off from the secondary which anastomose in the lymphatic cords. Look for lymph sinuses between the capsule and cortical follicles and along the primary trabeculæ. They are also found between the medullary cords and the trabeculæ. What is the nature of the medullary substance? Draw under low power showing general relations. Study a follicle and its germ-center under high power and make a drawing showing the arrangement of the cells.

Cells of Lymph Glands. Make preparations by drawing the freshly cut surface of a gland across the center of a clean slide. Fix in formalin vapor for ten minutes, or by heat, and stain in methylene blue and eosin. Dry and mount in balsam. Study under high power. Do you find lymphocytes? What varieties do you find? Sketch a cell of each type found.

The Spleen.

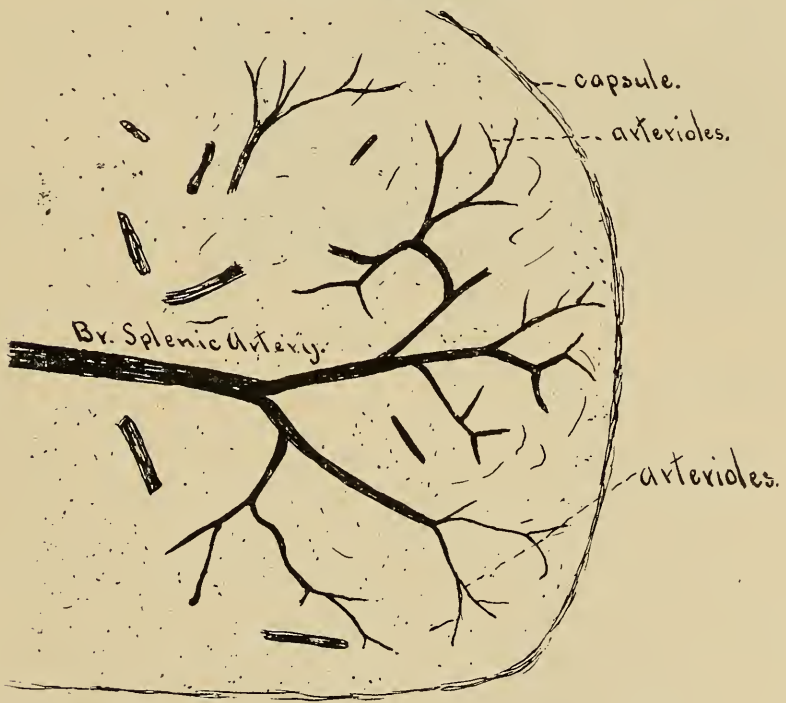


Fig. 26. Portion of injected Spleen of a Cat. x90.

Injected Spleen. The spleen of a cat was injected with carmine-gelatin, hardened in alcohol, imbedded in celloidin, and sec-

tioned. Dehydrate, clear, and mount in balsam. Study under low power. How do the arteries end? Sketch under low power. See Fig. 26.

Stained Spleen. Tissue from the spleen of a dog was fixed in a saturated solution of corrosive sublimate in normal salt solution, hardened in alcohol, imbedded in cellodin, and sectioned. Stain in hæmatoxylin and eosin and mount in balsam. Study under low power. What is the composition of the capsule? How is the

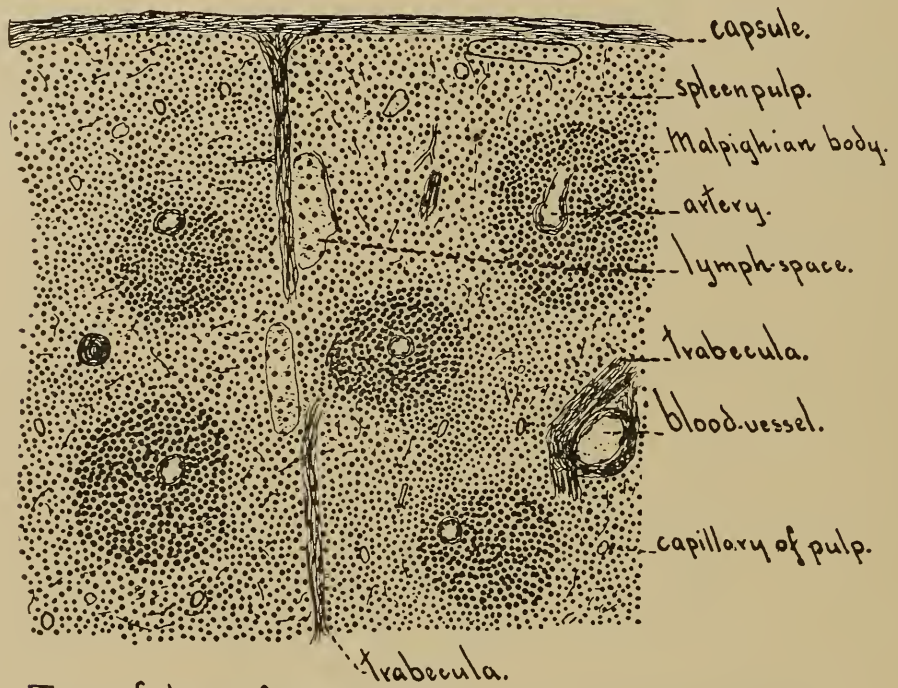


Fig. 27. Spleen of a Dog.

frame-work of the gland formed? What are the Malpighian corpuscles? What is their relation to the arterioles of the splenic artery? What is the nature of the splenic pulp between the Malpighian corpuscles? Make a drawing showing the general structural relations of the spleen. See Fig. 27.

Study the spleen pulp under the high power. In the reticulum should be found the following varieties of cells: (1) erthrocytes; (2) a few nucleated erythrocytes; (3) giant cells; (4) leucocytes, especially the mononuclear variety; (5) cells containing pigment probably derived from the hæmoglobin of broken-down red cells.

Nucleated Red Corpuscles in Spleen. The method of Dr. E. T. Williams gives good results. Take the fresh spleen of a hog and from the outer edge cut wedge shaped pieces which are drawn *very lightly* across the center of a clean slide making a thin smear. Fix for one minute in the following fluid :

Corrosive sublimate	.78 gram.
Sodium chloride	.28 gram.
Distilled water	30 c. c.

Stain in an aqueous solution of hæmatoxylin, and counterstain in eosin. Dry and study without a cover glass under high power. These preparations may be permanently mounted in balsam. Sketch a few erythrocytes, and erythroblasts if they are present.

The Tongue.

The apex of the tongue of a rabbit was fixed in bichloride of mercury, hardened in alcohol, and imbedded in paraffin. Fasten the section to the slide, remove the paraffin, stain in hæmatoxylin and Van Gieson's stain, dehydrate rapidly, clear in oil of cloves, and mount in balsam.

Study under low power. What forms of papillæ do you find? What kind of epithelium covers them? Do the cells covering the papillæ meet at the edges or overlap? Do you find a connective tissue within the papillæ? Do you find any glands? Where are they located? Are they serous or mucous? Study the musculature of the tongue. Is the muscle smooth or striated? How are the muscles arranged? Do you find any nerves? Sketch as seen under low power.

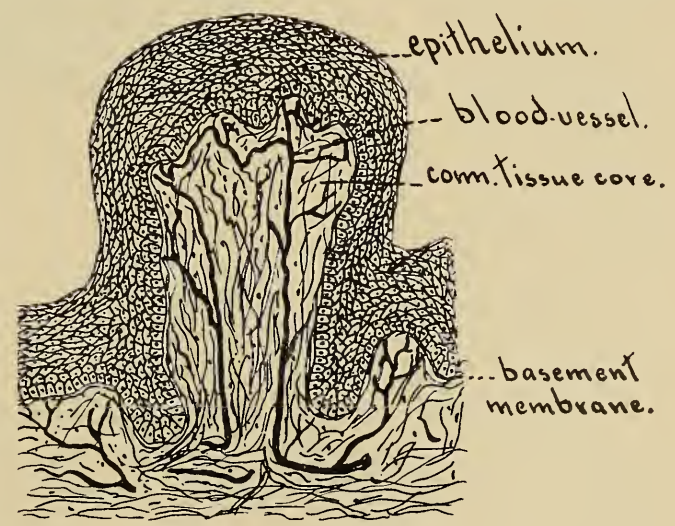


Fig. 28. Fungiform Papilla.

Fungiform Papillæ. Portions of the tongue of the dog containing fungiform papillæ were fixed and hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin, dehydrate, clear and mount.

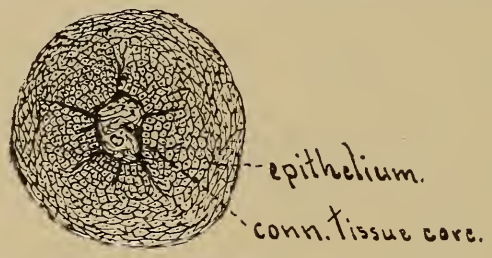


Fig. 29. T. S. Papilla Dogs Tongue.

Study under low power. Note the connective tissue core and epithelial covering of the papillæ. Do you find any taste-buds in the epithelium of the papillæ or in that surrounding it? Sketch papillæ as seen under low power. See Figs. 28 and 29.

Taste Buds. Portions from the tongue of the rabbit containing foliate papillæ were fixed, hardened, imbedded in paraffin, and sectioned at right angles to the folds. Stain in hæmatoxylin and eosin, after fixing to the slide and removing the paraffin, clear in oil of cloves and mount. Study first under low power. What is the location of the taste-buds? Do they extend through the epithelium? What type of glands do you find? What are they called? Sketch a portion as seen under low power. See Fig. 30.

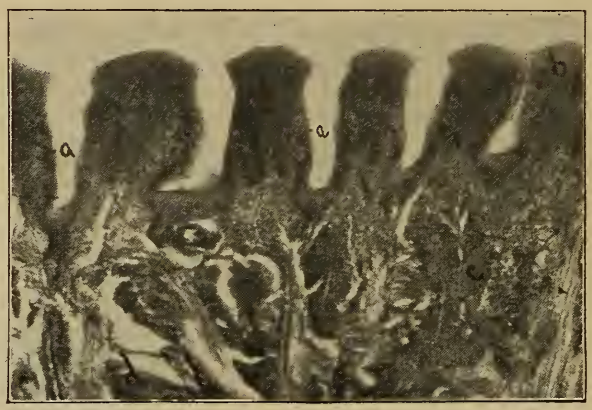


Fig. 30. Transverse section of the Foliate Papillæ of a Ribbit: *a*, taste-buds; *b*, papilla; *c*, glands; *d*, muscle.

Study the taste buds under high power. What is their form? Are they completely surrounded by epithelium? Sketch and show as much of the structure as you can.

Circumvallate Papillæ. Portions of the tongue containing circumvallate papillæ were hardened in alcohol, imbedded in celloidin and sectioned. Stain in hæmatoxylin and eosin, clear in Eycleshymer's mixture and mount in balsam. Study under low and high power as above and sketch as seen under low power, showing the structural features.

Alimentary Tract.

Œsophagus. The œsophagus of a dog was fixed in bichloride of mercury, hardened in alcohol, and imbedded in celloidin. Stain the section in hæmatoxylin and eosin, dehydrate, clear in Eycleshymer's mixture, and mount in balsam.

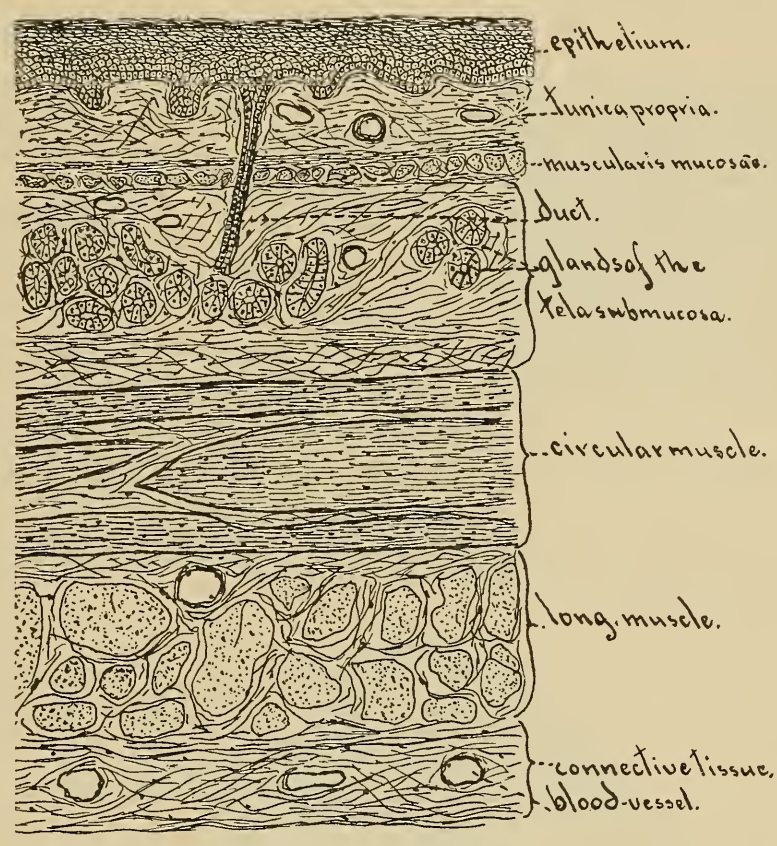


Fig. 31. Œsophagus of a Dog x 30.

Study under low power. What kind of epithelium do you find? Do you find a muscularis mucosæ? Study the structures found in the tela submucosa. What kind of glands do you find? Study the muscular layers. Is the muscle striated or non-striated? Sketch under low power and show the parts and general relations. See Fig. 31.

Cardiac Stomach at its Junction with the Œsophagus. Stain in hæmatoxylin and Congo red and mount in balsam. Study

under low and high power. Note changes in epithelium from stratified to simple columnar. Do you find glands in the tela submucosa? If so, what kind? What kinds of muscle do you find and how are they arranged? Make a drawing of the epithelium at the junction of the œsophagus and stomach.

The Stomach with Blood Vessels Injected. After injection through the aorta with carmine-gelatin, the stomach of a cat was hardened in alcohol, imbedded in celloidin, and sectioned. The sections are in clearing oil from which you will mount them in balsam. Study and sketch under low power showing the arrangement of the blood vessels. See Fig. 32.

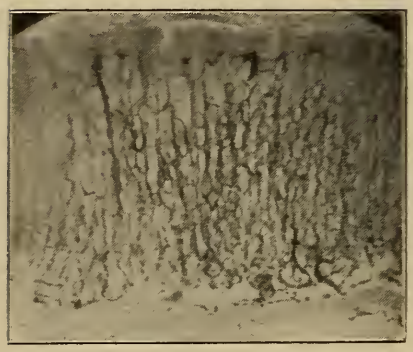


Fig. 32. Section of the Mucosa of a Cat's Stomach injected.

Cardiac Glands. Portions of the cardiac stomach of a dog were fixed in corrosive sublimate solution, hardened in alcohol, and imbedded in paraffin. Fix sections to slide, remove paraffin, and stain in hæmatoxylin and Congo red. Clear in oil of cloves and mount in balsam.

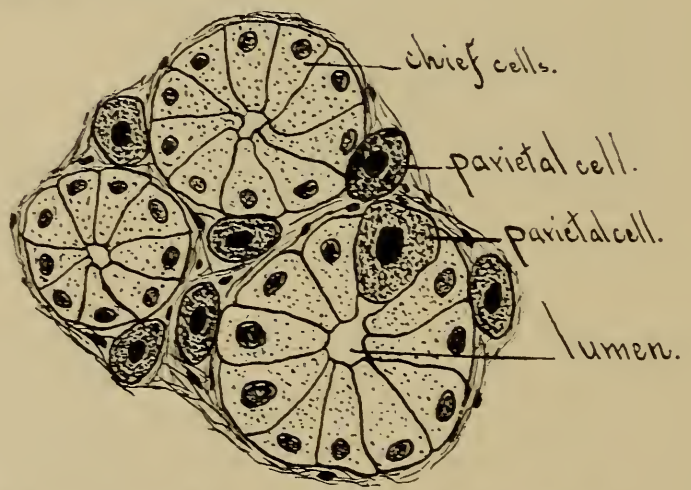


Fig. 33. Cardiac Glands Dog's Stomach.

Study under low power to get the general structural relations. What type of gland do you find? What is the nature of the tela

submucosa? Do you find any glands in it? How are the muscular tissues arranged? Sketch a portion as seen under low power.

Study the glands under the high power noting the chief or peptic cells and the parietal cells, oval in shape, with prominent nuclei, and stained with the Congo red. Sketch. See Fig. 33.

Pyloric Glands. Portions of stomach from the pyloric region were fixed in corrosive sublimate, hardened in alcohol, imbedded in

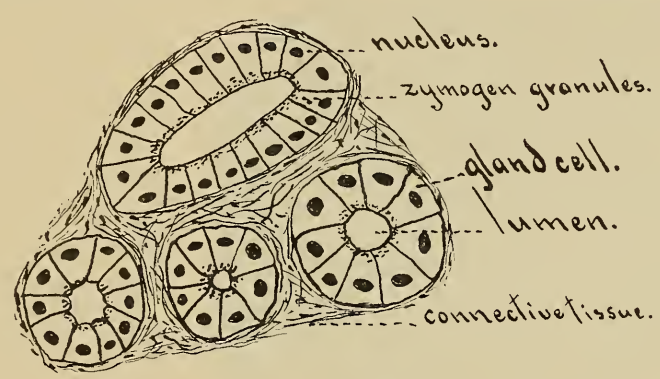


Fig. 33a. Pyloric Stomach Dog. Spongefed for 12 hours. Diagrammatic.

celloidin, and sectioned. Stain in hæmatoxylin and eosin, dehydrate, clear in oil of bergamot, and mount in balsam.

Study under low power. How do the ducts of the pyloric

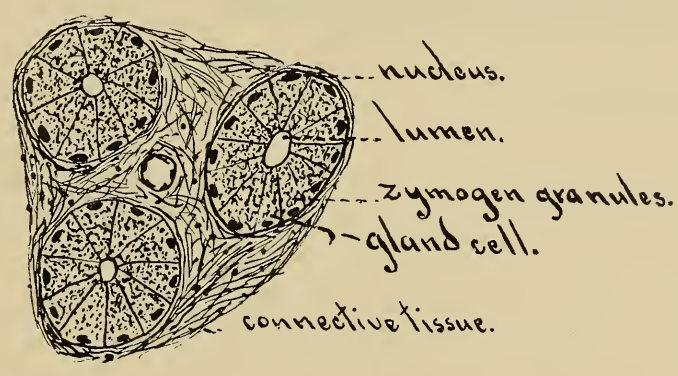


Fig. 33b. Pyloric Stomach Dog after 48 hours fasting. Diagrammatic.

glands differ from those of the cardiac glands? How do the secretory tubules differ? Compare the muscular layer with that of the

cardiac end. Do you find any goblet cells in either cardiac or pyloric sections? Sketch a portion under the high power to show the glandular epithelium and compare with the epithelium of the cardiac stomach.

Duodenum. Portions of the duodenum of a dog were fixed in bichloride of mercury, hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin and mount. Study under low power noting the coats and general relations. Do you find villi? Note the crypts of Lieberkühn and the glands of the tunica mucosa. Find Brunner's glands in the tela submucosa. Are they serous or mucous? Are they simple or compound? Tubular or saccular? Make a drawing using the low power so as to show the general structure. See Fig 34.

Fig. 34. Human Duodenum: *a*, solitary lymph nodule; *b*, diffuse adenoid tissue; *c*, Brunner's glands; *d*, glands of Lieberkühn.

Injected Ileum of a Cat. The injection mass used was carmine-gelatin and it was injected through the abdominal aorta. The intestines were hardened in alcohol, imbedded in celloidin, and sectioned. The sections are in clearing fluid and are to be mounted in balsam. Study and sketch under low power. See Fig. 35.

Small Intestine Stained. Portions of the ileum were fixed in bichloride of mercury, hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and Van Gieson's stain, dehydrate quickly, clear in Eycleshymer's mixture, and mount in balsam.

Study under low power. What is the shape and structure of

the villi? Note the glands of Lieberkühn. Do they extend through the mucosa? What kind of epithelium covers the villi? Are goblet cells present? Study the muscularis mucosæ. Note the vessels of the tela submucosa. Look for nerves in the submucosa and between the muscular layers. Do you find adenoid tissue? In what form does it occur? Note the tunica muscularis and the tunica serosa. What is the structure of the tunica serosa? Sketch a portion under the low power.

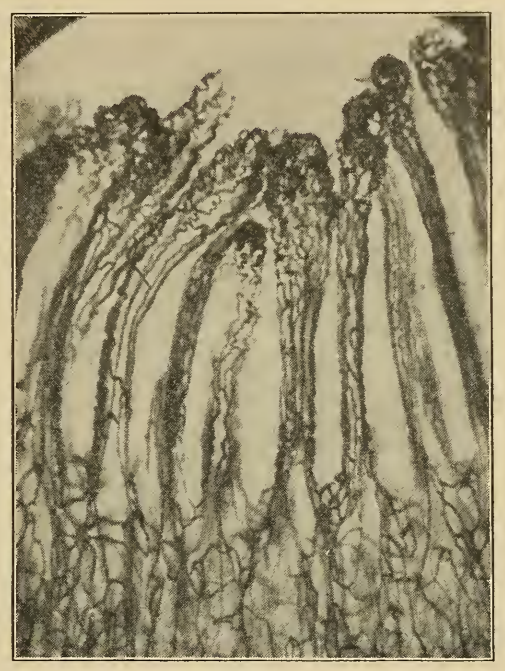


Fig. 35. Injected Intestine of a Cat.

Colon. Portions of the colon of a dog were fixed in bichloride of mercury, hardened in alcohol, and imbedded in paraffin. Fix the section to the slide, remove the paraffin, and stain with hæmatoxylin and eosin. Mount in balsam and study under low power. Are villi present? What glands are found in the tunica mucosa? What kind of epithelium do you find? Are goblet cells present? Do you find

Fig. 35a. Colon of Man: *a*, glands of Lieberkühn; *b*, tela submucosa; *c*, circular muscle layer; *d*, longitudinal muscle layer; *e*, muscularis mucosæ; *f*, blood-vessel.

diffuse adenoid tissue? Are solitary glands present? How is the muscular tissue arranged in the tunica muscularis? Sketch to show general structural relations and draw a gland of Lieberkühn under the high power. See Fig. 35a.

Vermiform Appendix. Portions of the appendix of a rabbit were hardened in alcohol, stained in Delafield's hæmatoxylin, and imbedded in paraffin. Fix to the slide, remove the paraffin with turpentine and xylol, and mount in balsam. Study and sketch under low power noting carefully the structural relations.

Digestive Glands.

Parotid.—Serous Type. Small pieces of the parotid gland of a dog were fixed and hardened in absolute alcohol, and imbedded in paraffin. Fix the section to the slide, stain in hæmatoxylin and

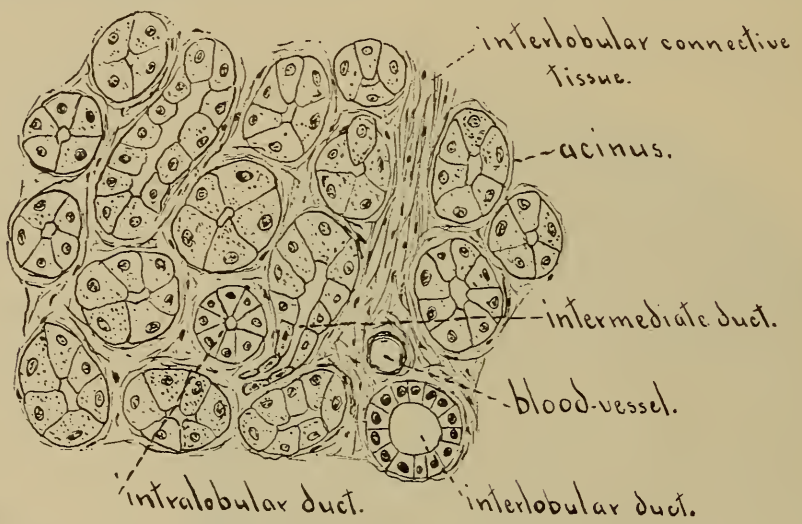


Fig. 36. Human Parotid Gland.

eosin, and mount in balsam. Study under low power and note the lobules which are held together by connective tissue. Of what structures are the lobules composed? Do you find any intralobular ducts? Study the acini under the high power. Is the protoplasm clear or granular? Are the cells in the resting or active condition? Do you find a basement membrane? Sketch a portion under the low power to show the general relations and a few of the acini

under the high power to show the epithelium of the acini and a duct. See Fig. 36.

Submaxillary of the Dog.—Mucous Type. The tissue was fixed in Flemming's solution, and imbedded in paraffin. Fix the section to the slide, and stain in Delafield's hæmatoxylin and Congo red. Dehydrate, clear, and mount in balsam. Study first under low power noting that the general structure is very similar to that of the parotid gland. Study the acini under high power. Is a basement membrane present? Are the cells clear or granular? What is the position of the nucleus? Look for the crescents of Gianuzzi or the demilunes of Heidenhain. What relation do they bear to the mucous cells of the acini? Are the cells of the crescents clearly defined? Sketch a small portion showing a duct, mucous acini and the crescents. See Fig. 37.

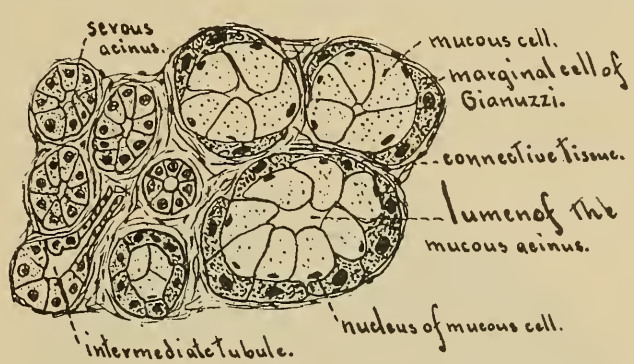


Fig. 37. Mucous and Serous Acini Human Submaxillary.

The Pancreas. Pieces of the pancreas of a dog were fixed in bichloride of mercury, hardened in alcohol, and imbedded in paraffin. Fix the section to the slide, remove the paraffin, stain, and mount in balsam.

Study under low power noting that the structure and general appearance is very similar to that of the parotid gland.

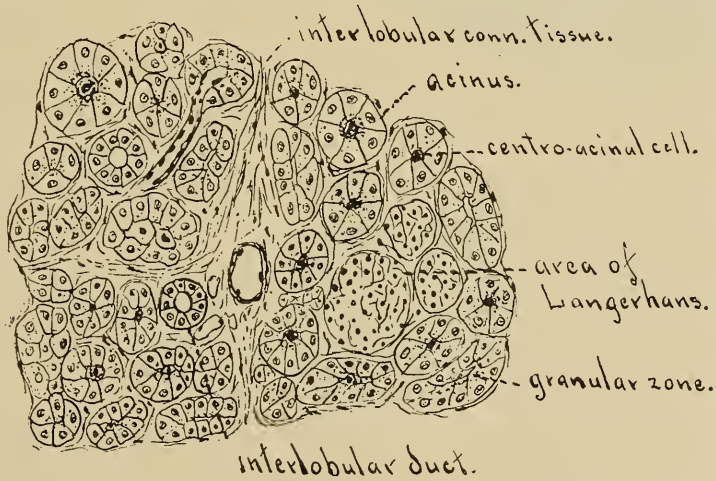


Fig. 38. Human Pancreas. x 250.

Study the acini under the high power. Is the protoplasm clear of granular? What is the position of the nucleus? Is a basement membrane present? Do you find the centro-acinal cells? Do you find the areas of Langerhans between the acini of the gland? How do they appear in contrast with the acini? Do you find any traces of ducts in them? Sketch an area of Langerhans with the surrounding acini as seen under high power. See Fig. 38.

The Liver with Blood-Vessels Injected. The liver of a dog was injected through the portal vein with carmine-gelatin, hardened in alcohol, imbedded in celloidin, and sectioned. The sections are in clearing oil and should be mounted in balsam.

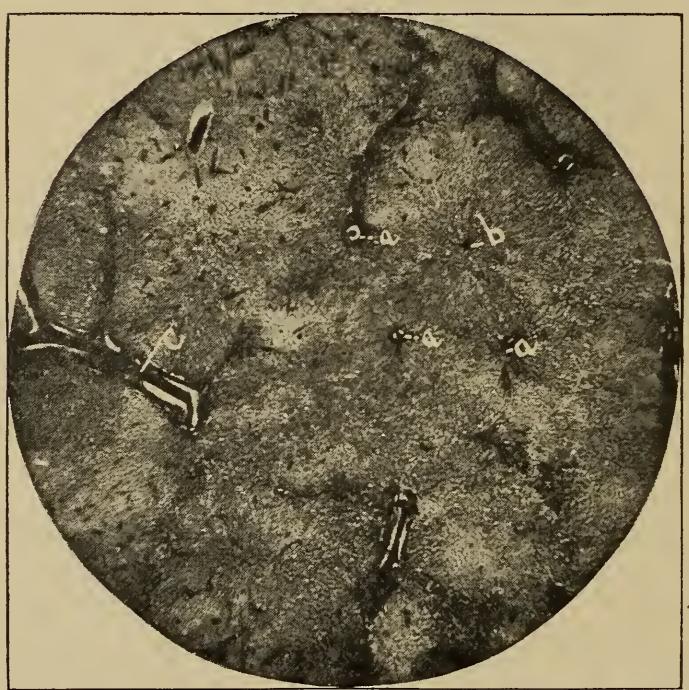


Fig. 39. Dog Liver Injected: *a*, interlobular veins; *b*, vena centralis lobulæ; *c*, branch of portal vein.

Study under low power noting the lobules and their arrangement. Between the lobules are the interlobular veins. Of what are they branches? Study the capillaries of the lobule noting that they originate from the interlobular and that they pass into the capillaries of the vena centralis lobulæ, the beginning of the hepatic system of veins. It is difficult to determine where the capillaries from the portal vein cease and those of the hepatic vein begin. By searching the section you should be able to find where a vena centralis lobulæ passes into a sublobular vein. Sketch under low power. See Fig. 39.

Injected and Stained Liver. Portions of liver as injected above were stained in Delafield's hæmatoxylin, imbedded in celloidin, and sectioned. Remove the sections from the clearing oil and

mount in balsam. Study as above, noting the relation of the hepatic cells to the capillaries, and sketch a small portion under high power.

Stained Pig Liver. Small pieces of pig liver were hardened in absolute alcohol and imbedded in paraffin. Fix the section to the slide, remove paraffin, and stain in hæmatoxylin and eosin, clear in oil of cloves, and mount in balsam.

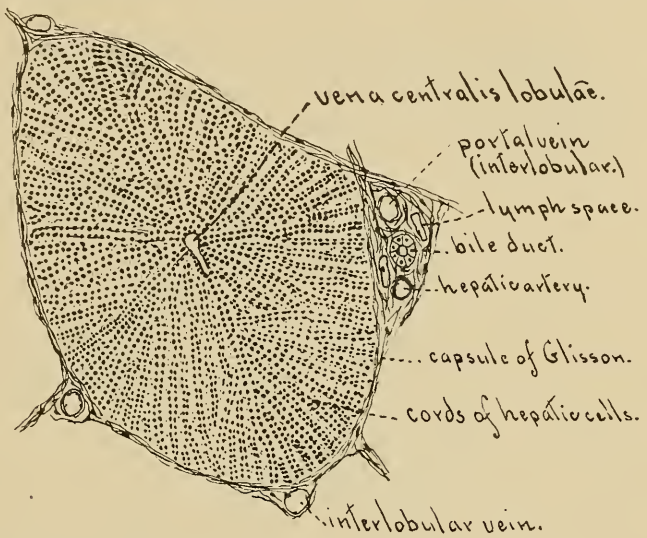


Fig. 40. Diagrammatic Sketch Lobule of Pig's liver x 90.

Study the lobules and general structure under low power. The pig's liver is well adapted for the study of the general structure since the lobules are very distinct. Find a portal canal containing a bile duct, hepatic artery, and portal vein. What is the position of the canal? Study the capsule of Glisson. Do you find lymphatic vessels in the canals? Draw a portal canal with portions of the adjacent lobules as seen under low power. See Fig. 40.

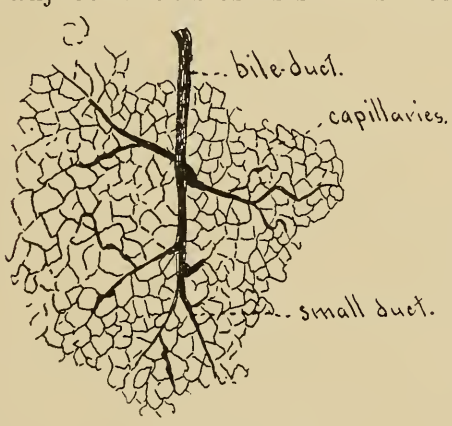


Fig. 41. Bile Capillaries and Ducts x 90.

Impregnated Liver. Oppel's Method. Pieces of liver were placed for three days in a solution consisting of four parts 3% solution of potassium bichromate, and one part of a 1% solution of osmic acid and then placed in a ¾ to 1% solution of silver nitrate where they remained several days, after which they were rapidly imbedded in celloidin, sectioned, and you will

mount and study them in the clearing oil. They may be mounted in *hard* balsam. The bile capillaries are stained black. Study their relations to the cells and sketch under high power. See Fig. 41.

The Kidney.

Kidney Macerated to Show Tubules. Place strips of the kidney of a cat or other animal in 25 to 30% hydrochloric acid for twenty four hours after which wash one hour in running water.

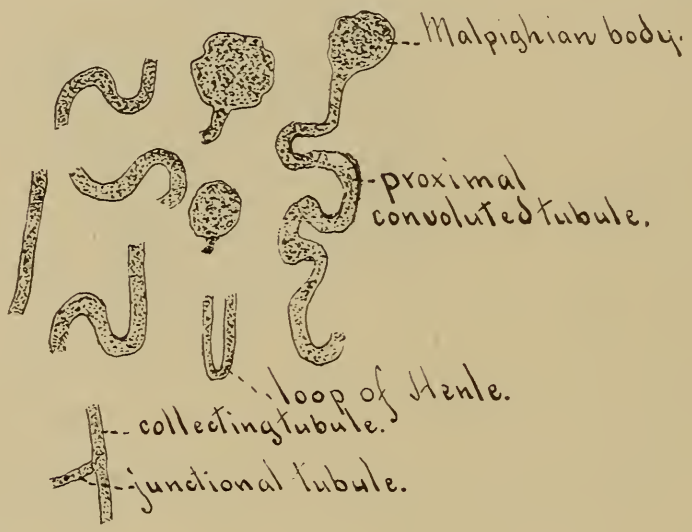


Fig. 42. Fragments of tubules Kidney Hog

Tease carefully and examine in 50% glycerin. The tubules will be broken up somewhat but by studying the preparation carefully under low power the parts of the tubule may be found. Sketch and label as many parts as you can recognize. See Fig. 42.

Kidney of a Cat Injected. The kidney of a cat was injected through the renal artery with carmine-gelatin, hardened in alcohol, imbedded in celloidin, and sectioned. Remove the sections from the clearing oil and mount in balsam.

Study under low power. Do you find portions of the arterial arch? Note the straight arteriæ rectæ extending into the medulla. Trace an interlobular artery from the arch into the cortex. Study the vasa afferentia as they terminate in the capillary loops or glomeruli. Trace the vasa efferentia as they leave the glomeruli and

form capillary net works about the convoluted portions of the tubuli uriniferi. Study the formation of the capillary network in the superficial part of the cortex. Draw under low power showing as many of these points as you can. See Fig. 43.

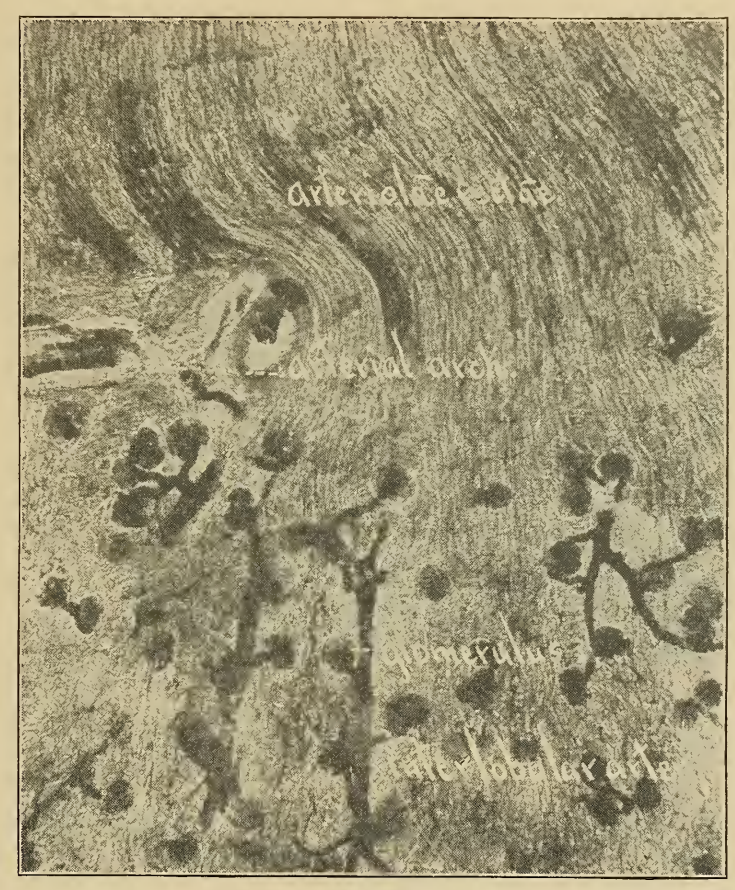


Fig. 43. Injected Kidney of a Cat x70.

Stained Kidney. A portion of the kidney of a cat was fixed in Carnoy's fluid for about five hours, the fluid was then washed out with absolute alcohol and the tissue was imbedded in celloidin, sectioned transversely, stained in iron-alum-hæmatoxylin, counterstained in acid-fuchsin, and the sections are now in clearing oil from which they are to be mounted in balsam.

Study under low power noting the capsule and the division into cortex and medulla.

Locate the medullary rays extending into the cortex from the base of the pyramid. Of what are they composed? Locate the columns of Bertini. What are they and why are they present? What is the labyrinth? Study the pyramids. Why do they have a striated appearance? Look for blood capillaries. Study the glomeruli. What is the relation of the glomerulus to Bowman's capsule? Sketch under low power to show the general structural relations. See Fig. 44.

Study the glomeruli under the high power. Study the differ-

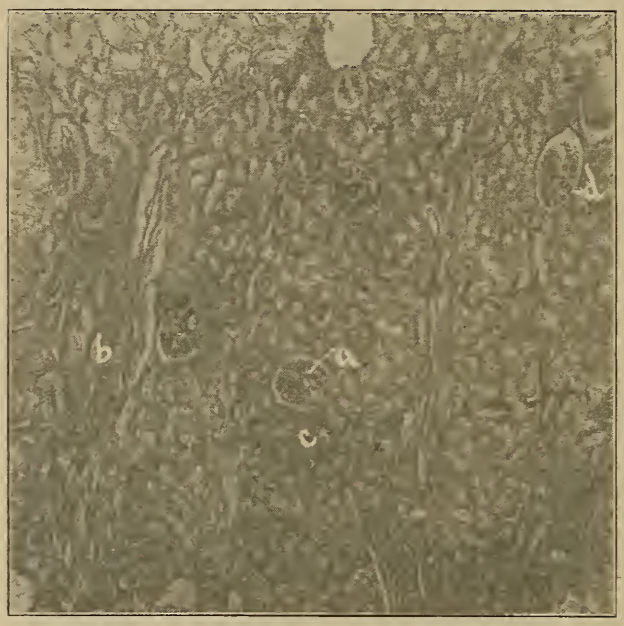


Fig. 44. Human Kidney: *a*. glomerulus; *b*, medullary ray; *c*, convoluted tubules; *d*, Bowman's capsule.

ent portions of the tubules under high power noting the variations in the character of the epithelium and sketch cells from each part to show the nature of the epithelium. Do you find a basement membrane? Study the capsule under high power. What is its composition? Notice the intertubular connective tissue, relatively small in amount in the normal kidney. Sketch a glomerulus and a portion of the surrounding tissue as seen under high power.

Longitudinal Section of the Kidney of a Fœtal Pig. The kidney of a fœtal pig was hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin, clear in Eycleshymer's mixture, and mount. Study as above, noting the development of the tubules and the relation of the glomerulus to Bowman's capsule. Sketch a portion.

Suprarenal Body.

The suprarenal body of a rabbit was hardened in alcohol and imbedded in paraffin. Fix the section to the slide, remove the paraffin, stain in hæmatoxylin and eosin, clear in oil of cloves, and mount in balsam.

Study under low power. Study the capsule and the septa passing into the body. Note the division into cortex and medulla. Study the cortex noting the zones of which it is composed. The outer zone is known as the glomerular zone since the cells are in groups separated by septa derived from the capsule. The middle zone has the cells arranged in columns or bundles of cells separated by the connective tissue and is called the fascicular zone. The

inner zone is composed of anastomosing cords of cells and is known as the reticular zone. Note that the medulla is composed of strands of cells usually pigmented and irregular in shape. What is the

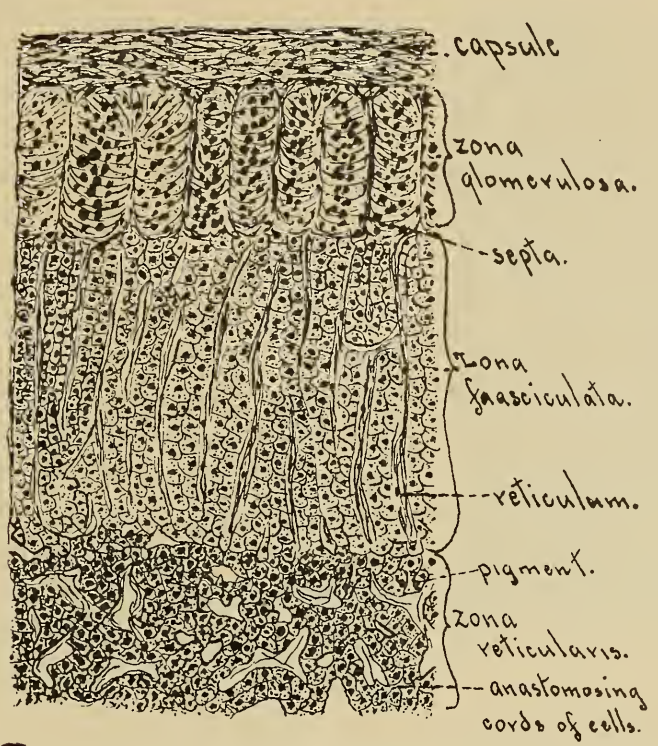


Fig. 45. Suprarenal Cortex Dog x 100. After B. von D.

nature of the spaces between the strands of cells? Study the blood vessels and nerves of the gland. Sketch a section under low power showing general structure. See Fig. 45.

Bladder and Ureter.

Collapsed Bladder. Pieces of the bladder of a cat were fixed and hardened in alcohol, imbedded in paraffin, and sectioned. Fix the section to the slide, remove the paraffin, stain in hæmatoxylin and eosin, clear in oil of cloves, and mount in balsam.

Study under low power. What kind of epithelium do you find? How many layers of cells are present? How do the cells of the various layers differ in form, and why do they so differ? Is diffuse adenoid tissue to be found in the tunica mucosa? Do you find glands in either the tunica mucosa or the tela submucosa?

How many muscular layers do you find in the tunica muscularis? Is the muscular tissue regularly or irregularly disposed? Do you find a tunica serosa? Study the section under high power and identify the structures fully, asking help of the instructor if necessary, and sketch a portion under the low power in order to show the relative thickness and relations of the layers. See Fig. 46.

Fig. 46. Collapsed Bladder of a Rabbit: *a*, connective tissue of tunica propria; *b*, transitional epithelium; *c*, muscle in longitudinal section; *d*, muscle in transverse section.

Distended Bladder. The bladder of a rabbit was distended with the fixing fluid and then placed in a quantity of the same fluid until fixed and hardened after which it was imbedded in paraffin, sectioned, and fixed to cover glasses. Remove the paraffin with turpentine and xylol, stain in hæmatoxylin and Van Gieson's stain, dehydrate quickly, clear, and mount. Study as above and compare the sections carefully noting the principal points of difference in the appearance.

Ureter Near Pelvis. Tissue was prepared as for the section of collapsed bladder and you will prepare and study in the same manner.

Ureter Near Bladder. The tissue was treated as above and the sections are to be stained and mounted. Compare the sections of ureter, especially the musculature. What kind of connective tissue do you find?

Male Reproductive Organs.

Testis. The testis of a rat was fixed in Flemming's solution, imbedded in paraffin, sectioned transversely, and fixed to cover-glasses. Remove the paraffin, stain in hæmatoxylin and eosin, dehydrate, clear in oil of cloves, and mount in balsam. Study under low power, noting first the capsula fibrosa or tunica albuginea which surrounds it. Trace the trabeculæ from the capsule into the gland

substance. Some fatty tissue may be found outside the capsule. Study the sections of the tubuli seminiferi, some of which are cut transversely and some obliquely, noting the layers of epithelial cells forming the walls. Note the epididymis at one side of the section. How is the canal of it lined? Fat globules may be found stained black with the osmic acid. Sketch a portion under low power to show the general relations. See Fig 47.

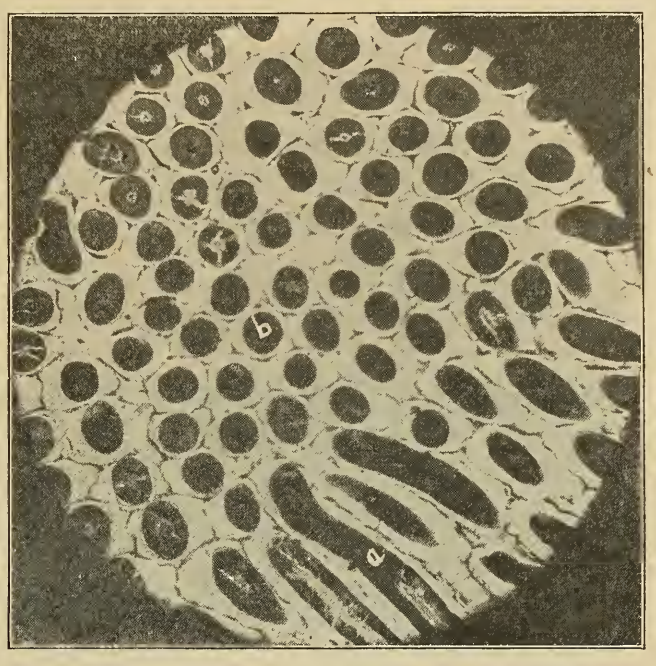


Fig. 47. Testis of a Rat under low power: *a*, tubule cut longitudinally; *b*, tubule cut transversely; *c*, intertubular connective tissue.

Fig. 48. Single Tubule Rat's Testis highly magnified. The lumen of the tubule is seen at the center and the sperm cells may be seen in radial rows.

Study the tubules under high power. By moving the section tubules in different stages of development may be found. Study the intertubular tissue carefully. Do you find a distinct basement membrane? What is the nature of it? Within the membrane you find the layer of spermatogones and the bases of the supporting cells or cells of Sertoli. The second layer

is that of the sperm mother-cells or spermatocytes. Next in order are several layers of smaller cells, the spermatoblasts or spermatids, which become sperm cells without further division. Can you find spermatids that have fused with the cells of Sertoli? Study various tubules very carefully and sketch several to show as many of the stages in development as possible. See Fig. 48.

Testis. Compare with the above sections prepared as above and stained in iron-alum-hæmatoxylin. Study the tubules of this

Fig. 49. Transverse Section of the Penis of a Dog: *a*, urethral lumen; *b*, epithelium; *c*, corpus spongiosum surrounding the urethra; *d*, corpora cavernosa showing some ossification; *e*, tunica albuginea; *f*, blood-vessel.

section under high power as this stain is very satisfactory for the nuclear structures.

Penis. The penis of a dog was fixed and hardened in alcohol, imbedded in celloidin, and sectioned transversely. Stain in hæmatoxylin and Van Gieson's stain, dehydrate rapidly, clear in Eycleshymer's mixture, and mount in balsam.

Study first under low power noting the three masses of erectile tissue. The corpora cavernosa have a strong, dense tunica albuginea surrounding them. The corpus spongiosum surrounds the urethra. Note the septa and trabeculæ derived from the tunica albuginea. What tissues are found in the sheath and septa? Do the spaces formed by the septa and trabeculæ communicate with each other? Are the spaces lined with endothelium? Note the blood vessels. Study the urethra. What kind of epithelium in it? Do you find any glands? Study the blood vessels of the submucosa and the muscular tissue of the urethra. Sketch as seen under the low power and label the parts. See Fig. 49.

Prostate Gland. The prostate gland of a dog was fixed and hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin, dehydrate, clear in Eycleshymer's mixture, and mount in balsam.

Study under low power. Notice the structure of the capsule noting the large proportion of smooth muscle in it. Study the trabecular framework. What type of gland do you find? What kind of epithelium lines the tubule? Is it a serous or mucous gland? Is a basement membrane present? Find the duct emptying into the urethra. What kind of epithelium forms the lining of the duct? Sketch a portion under low power to show the general structural relations and a portion of a gland as seen under high power.

Cowper's Gland. Portions of the gland were fixed, hardened, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin, and mount in balsam. Study the general structural features under low power. What type of gland as to form? As to secretion? Are crescents of Gianuzzi present? What kind of epithelial lining do the ducts possess? Look for larger ducts lined by two or three layers of cells. Sketch a portion of the gland as seen under high power.

Female Reproductive Organs.

Ovary. The ovary of a dog was fixed in Flemming's solution, hardened in alcohol, and imbedded in paraffin. Fasten the section to the slide, remove the paraffin, stain in hæmatoxylin and eosin, clear in oil of cloves, and mount in balsam.

Study first using the low power. Note the division into the cortical and medullary portions. The larger vessels are found in the medulla. Study the germinal epithelial layer. The framework of the ovary is known as the stroma. What is its composition? In the cortex you should find the Graafian follicles in the different stages of development. The simple primitive follicle has but a single layer of epithelial cells surrounding the egg-cell and surrounded by stroma. Find such follicles. The fully developed follicle has a rather dense stroma, the theca folliculi, surrounding it and instead of a single layer of cells lining it, it will have several layers forming the membrana granulosa. Can you distinguish the fibrous tunica externa of the theca? Note that this layer blends closely with the tunica interna which is more vascular than the externa and has more cells. One portion of this membrana granulosa becomes thickened forming the discus proligerus surrounding the egg-cell. Some of the cells have disappeared forming the antrum or cavity of the follicle, containing the liquor folliculi. What causes the precipitate found in the antrum in the sec-

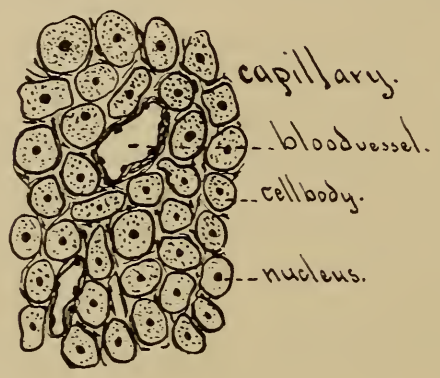


Fig. 50. - Corpus luteum x 400.

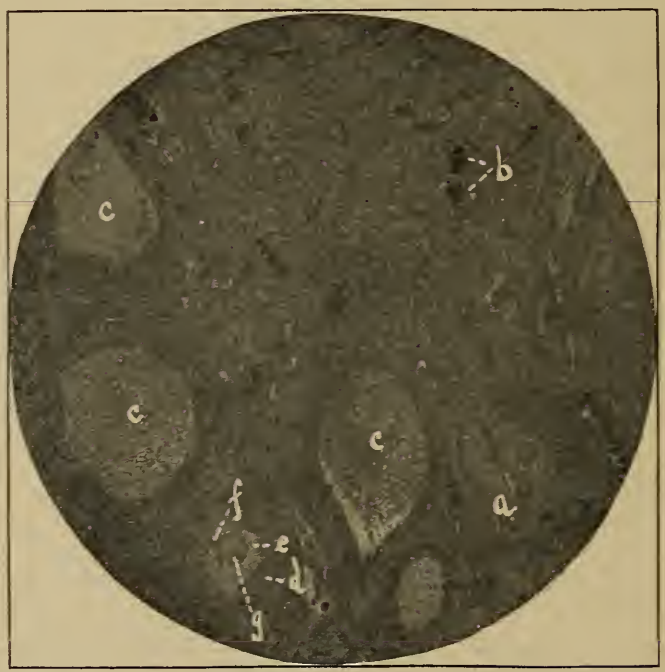


Fig. 51. Ovary of a Dog: *a*, stroma; *b*, blood-vessels; *c*, empty Graafian follicles; *d*, membrana granulosa; *e*, antrum of the follicle; *f*, discus proligerus; *g*, ovum.

tions? Study the various stages of development between the primitive follicle and the fully developed follicle. Do any follicles have more than one egg-cell? Do you find a corpus luteum composed of relatively large cells having small nuclei? If so, study carefully. See Fig. 50. Make a sketch under low power so as to show the general features of the ovary and sketch several follicles under the high power to show the stages of development. See Fig. 51.

Ovary of a Very Young Animal. The ovary of a young kitten was treated as above. Stain in hæmatoxylin and eosin and mount. This is designed to show the development of the egg-tubes from the germinal epithelium.

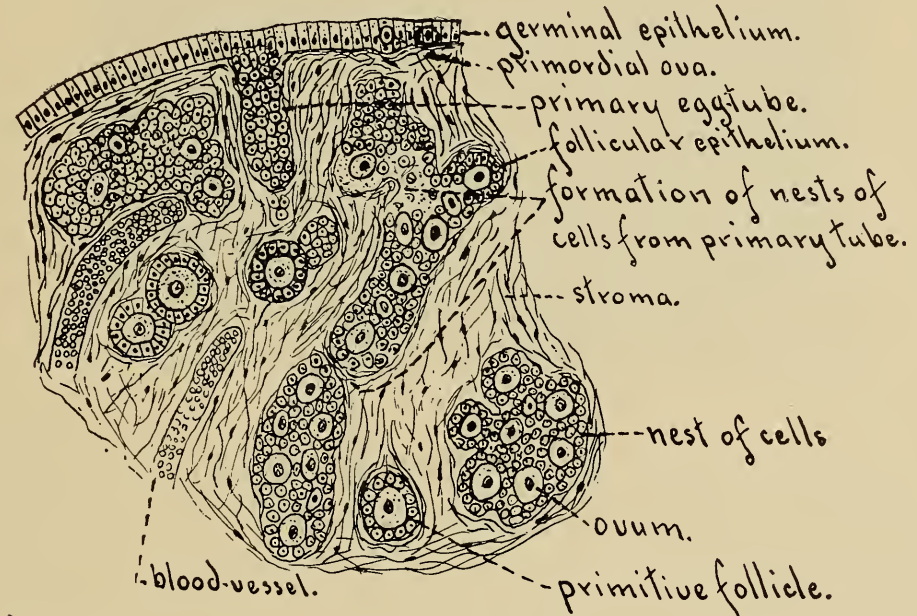


Fig. 52. Ovary of a very young Child. (Waldeyer.)

Study under low and high power. Find a downward growth of cells forming the egg-tube. Note that it is simply a downward proliferation of the cells from the layer of germinal epithelium into the stroma. Are all of the cells of the tube alike? Study other tubes and note that certain cells form the ova and others form the follicular epithelium surrounding them. Search for a tube which has been penetrated by connective-tissue septa so as to divide it into somewhat distinct epithelial nests. The connective tissue later

forms the theca folliculi surrounding the follicle. Sketch a portion of the section showing as many of these points as possible. See Fig. 52.

Fig. 53. Uterus of a Dog.

Uterus. Portions of the uterus were fixed in a saturated solution of corrosive sublimate in normal salt solution, hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and Van Gieson's stain, dehydrate rapidly, clear in Eycleshymer's mixture, and mount in balsam.

Study under low and then under high power. How many layers do you find? What kind of epithelium in the mucosa? What is the nature of the tunica propria? Are glands present in the mucosa? Of what type are the glands? What kind of muscle in the tunica muscularis? How many layers? What do you find between the layers? Study the vessels of the tunica muscularis. Study the tunica serosa which presents the usual characteristics of serous membranes. Sketch a portion under low power. See Fig. 53. Study and sketch as much of a gland as possible as seen under the high power.

Vagina. The vagina of a dog was fixed and hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin, and mount in balsam.

Study under low power. What kind of epithelium do you find? What are the characteristics of the tunica propria? Do you find any adenoid tissue in the mucosa? Are glands present? Do lymph nodules occur? Is a tela submucosa present? Study the tunica muscularis. Does it have distinct layers? Study the outer fibrous coat. Sketch as seen under low power so as to show the general structural features. Study under high power.

Mammary Glands.

Portions of mammary glands were hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin, dehydrate, clear, and mount.

Study under low power. The lobules of the gland are composed of acini united by fibrous tissue. Search for and study the ducts. Study under high power. What variety of epithelium in the lining of the acini? What is the position of the nuclei? What do you find in the lumen of an acinus? How do you account for the presence of fragments of epithelial cells? Study the epithelium carefully and then sketch a portion of the gland as seen under high power.

The Skin and its Appendages.

Pieces of human skin were fixed in alcohol, and imbedded in paraffin. Fix the section to a slide, remove the paraffin and stain, one in hæmotoxylin and eosin, and one in hæmatoxylin and Van Gieson's stain, clear in oil of bergamot and xylol, and mount in balsam.

Study under low and high power. The layers of the epidermis are as follows, beginning with the outer one: (1) Stratum corneum or the horny layer. Are the cells well defined? Do they possess nuclei? (2) Stratum lucidum. Why so called? Of what form of cells is it composed? Is this layer granular? (3) Stratum granulosum. What is the form of cells? What is the nature of the granules in the cells of this layer? (4) A layer of stratified squamous epithelium the deepest cells of which are columnar in

form. Note the transition in form of the cells as you go toward the surface. Why does the form of the cell change?

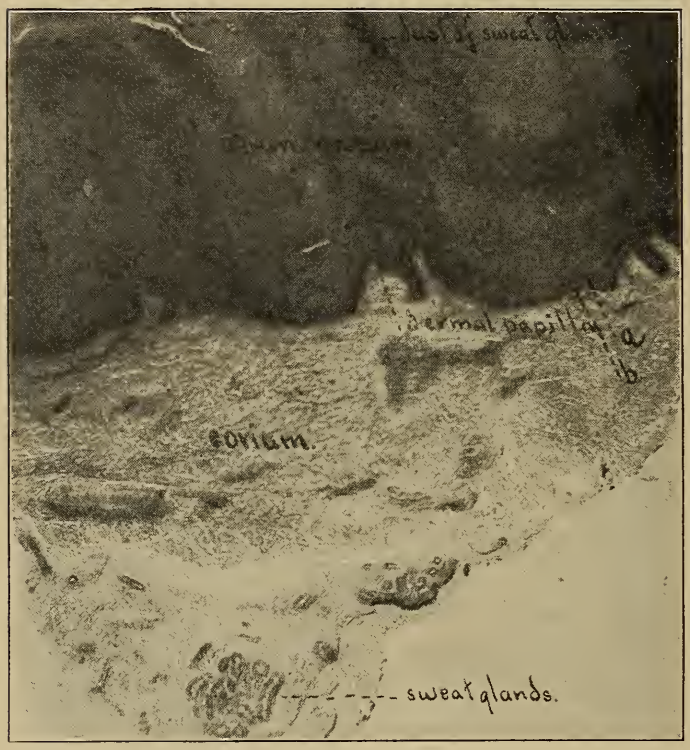


Fig. 54. Transverse Section of Skin from the Human Heel showing the great development of the stratum corneum; *a*, stratum lucidum; *b*, stratum Malpighii.

Study the dermis noting its general structure. Can you distinguish the papillary and reticular layers? What is the general direction of the fibers in the pars reticularis? Note that the fibers of the pars papillaris are somewhat finer and hence the tissue is somewhat more dense than in the pars reticularis. Study the papillæ. Study the glands of the skin under low and high power. Note their location and relations. Study the subcutaneous tissue. Draw and label a portion of the section as seen under the low power so as to show general relations. Sketch a few tubules of the sweat glands as seen under high power. See Fig. 54.

The Scalp. Portions of human scalp were hardened in alcohol, imbedded in celloidin, and sectioned transversely. Stain in hæmatoxylin and eosin, clear in Eycleshymer's mixture, and mount in balsam.

Study under low power and then under high power paying especial attention to the hair and follicles and to the sebaceous glands. Sketch a hair and follicle as seen under high power, and label all the parts. See Fig. 55. Make a sketch of a sebaceous gland as seen under low power and show its relation to the hair follicle.

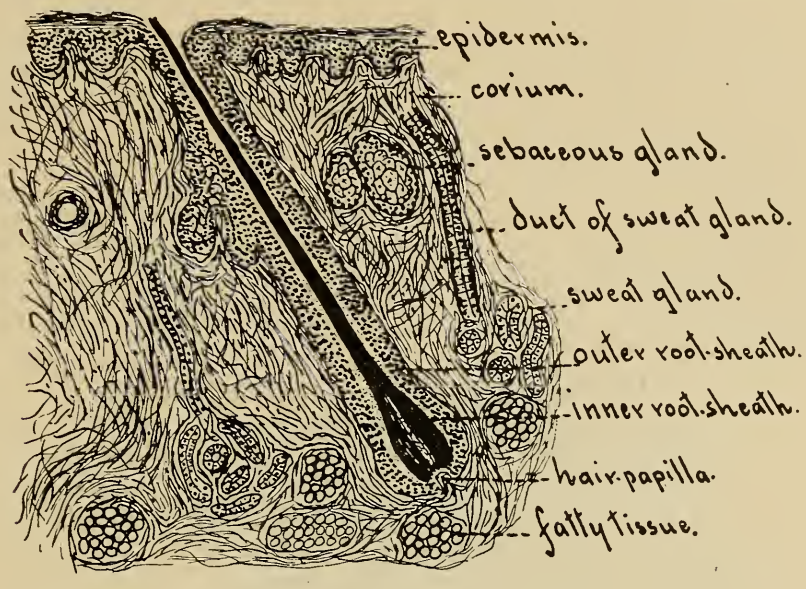


Fig. 55. T.S. Scalp of Man.

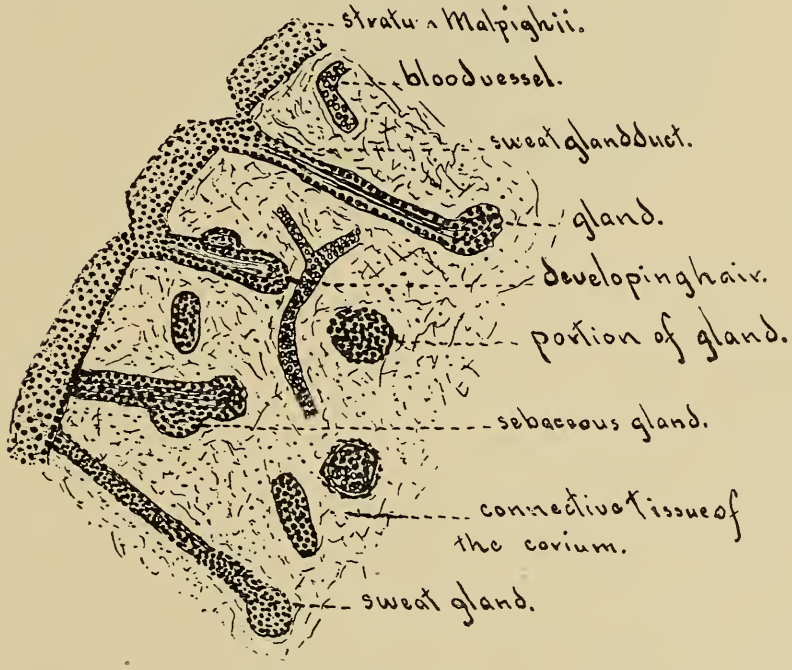


Fig. 56. T.S. Scalp of Human Fœtus.

Fœtal Scalp. Portions of fœtal scalp were hardened in formalin, imbedded in celloidin, and sectioned transversely. Stain in hæmatoxylin and Van Gieson's stain, dehydrate rapidly, clear in

Eycleshymer's mixture, and mount in balsam. Study carefully, noting the development of the hair glands, and the relation of the glands to the other structures of the scalp. Sketch a portion as seen under low power. See Fig. 56.

Scalp Showing Hairs in Transverse Section. Portions of scalp were imbedded in celloidin and sectioned tangentially. Study the follicles under high power and note the layers. Note the division into cortical and medullary portions. Study each carefully.

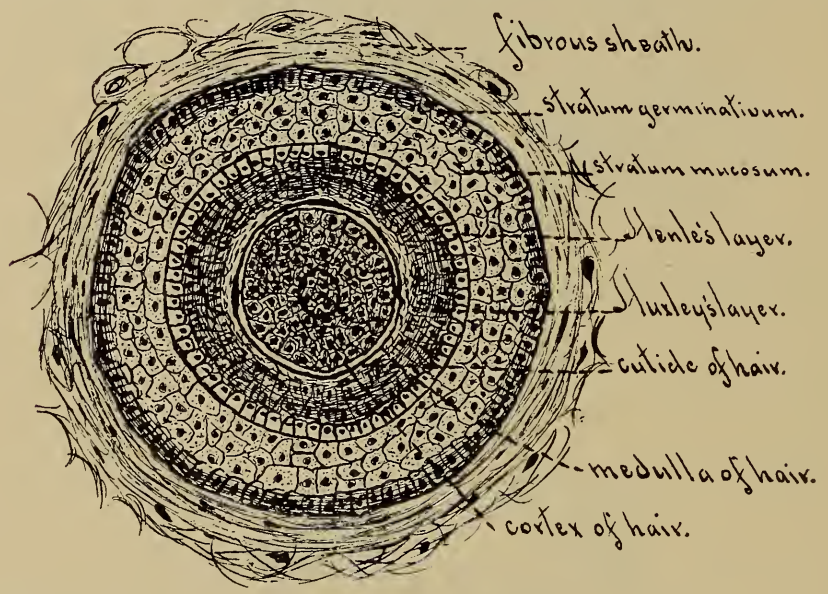


Fig. 57. T.S. Human Hair and Follicle x 300.

Study the layers of the root sheath. The inner sheath has three concentric layers of cells ; the inner cuticle surrounding the hairs, the middle layer of Huxley composed of two layers of cells, and the outer single layer of clear cells or layer of Henle. The outer root-sheath has in its inner portion several layers of prickle cells composing the stratum Malpighii, the outer layer of which is columnar in form. Next in order is the glassy membrane, then the circular connective tissue, and then comes the looser outer connective tissue with the bundles longitudinally placed. Sketch and label as seen under high power. See Fig. 57.

Development and Structure of the Nail. The thumb of a fœtus was hardened in formalin, imbedded in celloidin, and sectioned longitudinally. Stain in hæmatoxylin and eosin, clear in

oil of bergamot, and mount in balsam. Study the section under low power. See Fig. 58. Sketch. Study under high power remembering that the nail itself represents an enormously developed

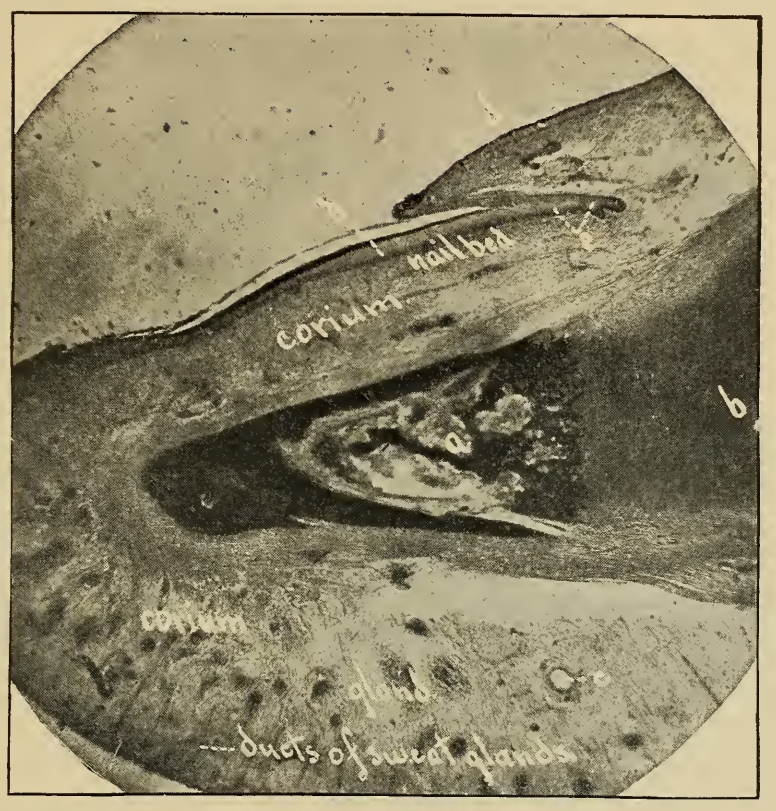


Fig. 58. L. S. Thumb of a Fœtus x 30 : *a*, ossifying bone of the terminal phalanx ; *b*, hyaline cartilage ; *c*, blood-vessel ; *d*, stratum Malpighii ; *e*, matrix of nail.

stratum lucidum. Study the cells of the nail. The nail bed is composed of the stratum Malpighii of the skin and the corium. The connective tissue fibers are somewhat coarser than they are in the corium of the skin. Study the matrix, the posterior portion of the nail-bed.

Larynx, Trachea, and Lung.

Larynx. The larynx of a rabbit was fixed and hardened in formalin, imbedded in celloidin, and sectioned transversely. Stain in hæmatoxylin and eosin, and mount in balsam.

Study under the low power. What kind of epithelium do you find in the tunica mucosa ? Are goblet cells present ? Study the

tunica or lamina propria. Are papillæ present? Do you find any diffuse adenoid tissue? Study the tela submucosa. Look for mucous glands. Study the cartilages. What variety of cartilage is found in the larynx? Study the perichondrium. Study the distribution of the blood vessels. Sketch a portion as seen under low power naming the parts.

Trachea. The trachea of a cat was fixed and hardened in alcohol and imbedded in paraffin. Fix a section to each of two slides, remove the paraffin and stain, one in hæmatoxylin and eosin, and the other in Weigert's Fuchsin-resorcin for about half an hour, wash in alcohol, treat with absolute alcohol, clear with xylol, and mount in balsam. The elastic fibers should be stained dark blue and the nuclei should stain but little if at all.

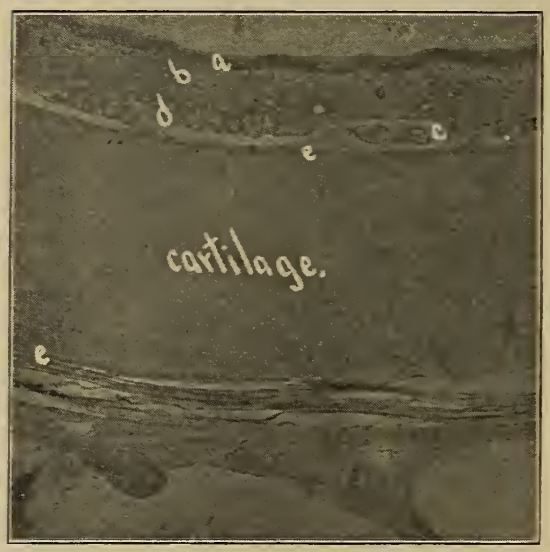


Fig. 59. Trachea: *a*, epithelium: *b*, tunica propria; *c*, tela submucosa: *d*, glands; *e*, perichondrium.

Study under low and high power. The first preparation shows the general structure very nicely while the second shows the distribution of the elastic tissue. What is the general direction of the elastic fibers of the lamina propria? What type of glands do you find and where are they located? Make a sketch under low power, using both sections, so as to show the structure of the trachea. See Fig. 59.

Lung. (a) Portions of the lung of a cat were fixed in 10% formalin, imbedded in celloidin, and sectioned. The lung of a larger animal is better for study and the portions should be kept entirely submerged in the fluid while undergoing fixation. Stain in hæmatoxylin and eosin, dehydrate, clear in oil of bergamot, and mount. Study under low power noting the sections of bronchi, blood-vessels and the lung tissue which is as seen in a collapsed lung. Compare your section with Fig. 60 and identify the parts.

Study one of the larger bronchioles. What is the nature of the epithelial lining? Are goblet cells present? Do you find any glands in the mucosa? If so, what kind? What is the next tissue

Fig. 60. Lung of a Cat: *a*, bronchiole; *b*, blood-vessel; *c*, respiratory bronchiole: *d*, lung tissue.

beneath the mucosa? Do you find any cartilage? Does it occur as more or less complete rings or as plates somewhat irregularly disposed? Do you find an outer fibrous covering? Do you find branches of the pulmonary artery in relation to the bronchiole? Do you find a branch of the bronchial artery? Sketch the bronchiole and a little of the surrounding lung tissue as seen under low power.

Study a small bronchiole as above. How does it differ in structure from a larger bronchiole? Sketch as above. Study Fig. 61 to learn the general relation of the blood-vessels to the lung tissue and then find a respiratory bronchiole and study carefully. Look for the capillaries often containing blood corpuscles. Study the epithelium of the air-cells. Are all of the cells alike? How

do they differ? Sketch a respiratory bronchiole and a portion of the epithelium of the air-cells as seen under high power.

(b) The lung of a dog was distended with a 10% solution of formalin after which the thorax was opened, the lungs removed and

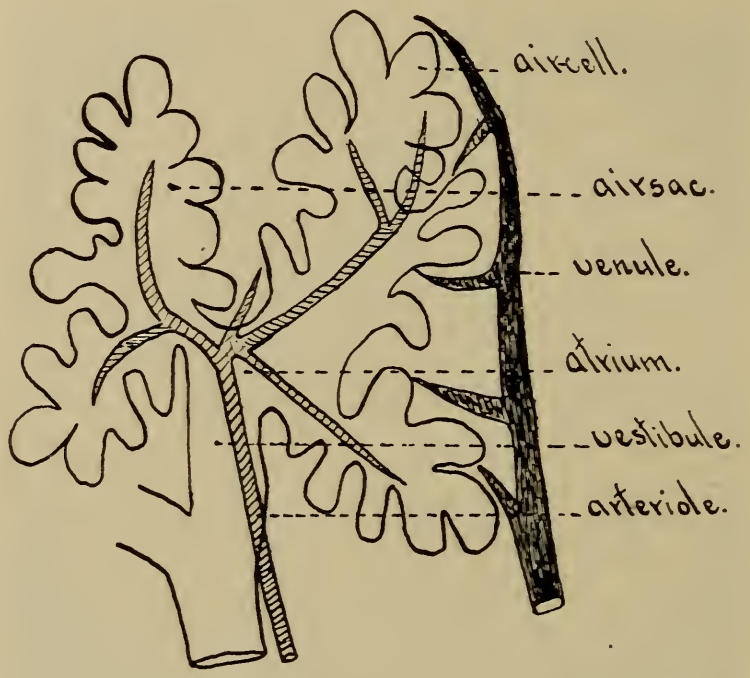


Diagram of ending of a Bronchial Tube. (Miller.) Fig. 61.

suspended in 10% formalin for a few days, after which portions were dehydrated, imbedded in celloidin, and sectioned. Stain and study as above noting the appearance of the distended lung and

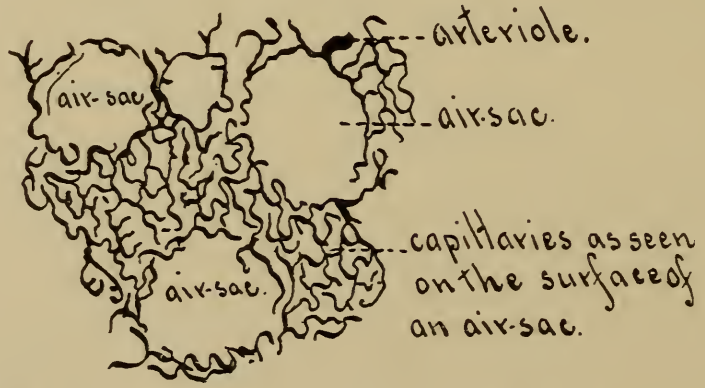


Fig. 62. Capillaries injected. Lung of a Cat.

especially the epithelium of the air-cells. Sketch a portion of the lung tissue to show the epithelium and compare with the above.

(c) Injected lung. The lung of a cat was injected with carmine-gelatin through the pulmonary artery, hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin and mount. Study and then make a sketch of a portion to show the distribution of the pulmonary artery. See Fig. 62.

(d) Elastic tissue of the lung. Lung tissue was hardened in alcohol and imbedded in paraffin. Fix a section to the slide and stain as follows: Place sections for twenty-four hours in orcein, (1-10 gm. of orcein in 20 c. c. of 95% alcohol, and 5 c. c. of distilled water, to which a solution is added consisting of 20 c. c. 95% alcohol, 1-10 c. c. hydrochloric acid, and 5 c. c. distilled water), differentiate in acid-alcohol for fifty or sixty seconds. Rinse in alcohol, and stain the nuclei with an alcoholic solution of methylene blue, rinse in alcohol, dehydrate in absolute alcohol, clear in xylol, and mount in balsam. Nuclei stain blue and elastic fibers a deep brown. Study carefully and sketch a portion to show the distribution of the elastic fibers.

The Thyroid Gland.

Portions of the thyroid of a dog were fixed in bichloride of mercury, hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin.

Another portion of the gland was fixed in Flemming's solution and imbedded in paraffin. Fix a section to the slide remove the paraffin with turpentine, and add xylol and the grades of alcohol down to water, and then place them in 2.5% aqueous solution of ammonium sulphate of iron for

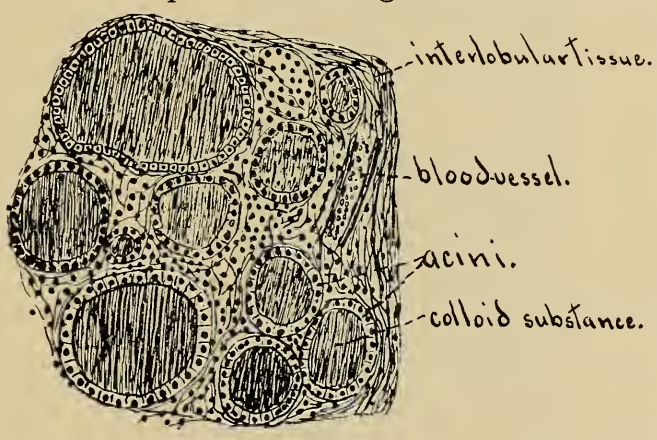


Fig. 63. Thyroid Gland of Dog x 400.

about five hours. Rinse in water and stain in the following solution of hæmatoxylin: Hæmatoxylin crystals, 1 gm.; absolute alcohol 10 c. c.; distilled water 90 c. c.; diluted when used with an equal volume of distilled water. They should be stained for twenty-four hours, rinsed in water, and again placed in ammonium sulphate of iron solution until the excess of stain is removed, after which they are washed carefully in distilled water, dehydrated, cleared in xylol, and mounted in balsam. This is known as Heidenhain's Iron-Hæmatoxylin Stain.

Study the first section under low power. Note the fibrous capsule investing the gland. What is the shape of the alveoli? How are they lined? Study the colloidal substance. Does it stain uniformly and what is its composition. Study the second section as above, compare with the first, and then sketch a few alveoli to show their appearance under the high power. See Fig. 63.

Huber recommends fixing with Flemming's fluid and staining with the Ehrlich-Biondi mixture. This stain serves to differentiate the chief from the colloidal cells. The chief cells do not stain, while the colloidal cells are red with green nuclei. This method has given good results with us.

The Eye.

Cornea. The eye of a man was removed, cleaned of superfluous fat and connective tissue, placed in Flemming's solution and divided into anterior and posterior halves. After fixing the lens was removed and one fourth of the anterior half was stained in hæmatoxylin, dehydrated, cleared, and imbedded in paraffin. Fix your section to the slide, remove the paraffin with xylol, and mount in balsam.

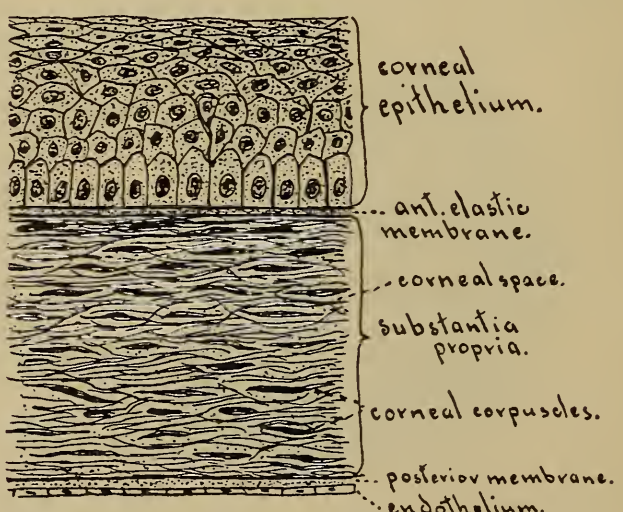


Fig. 64. Human Cornea x 450.

Study the cornea noting carefully the following layers: (1)

The corneal epithelium. How many layers of cells in it and of what variety are they? (2) The second layer is the anterior elastic membrane. What can you determine as to its structure? Does it extend to the sclera? (3) The third layer is the substantia propria or corneal ground-substance. Study the structure carefully noting that it seems to be composed of fibrous tissue, both in bundles and lamellæ, between which cells or corneal corpuscles are found in the corneal spaces. Is the substantia propria continuous with the connective tissue of the sclera? (4) The posterior elastic membrane. What is its structure? (5) The endothelium of the anterior chamber. Sketch a portion as seen under high power. See Fig. 64.

The Sclera. Study carefully, especially near and at its junction with the cornea. Of what layers is it composed? Study the canal of Schlemm. Study also the relations of the iris and ciliary processes as shown in the section. Sketch to show the general structural relations.

The Choroid Coat. Portions of the posterior half of the eyeball were imbedded in paraffin, and sectioned transversely. Fasten to the slide, remove the paraffin, stain in hæmatoxylin and eosin, and mount in balsam. Study carefully under low and high power and try and distinguish the following layers from without inward: (1) The lamina suprachoroidea. What is its composition? To what layer of the sclera is it joined? Are pigment cells present? Do you find the perichoroidal lymph-spaces? How are they formed and with what are they lined? (2) The lamina vasculosi. What is its structure? How does it differ from the above in structure? How are the blood vessels distributed? Locate and study the boundary zone. (3) Lamina choriocapillaris. What is its

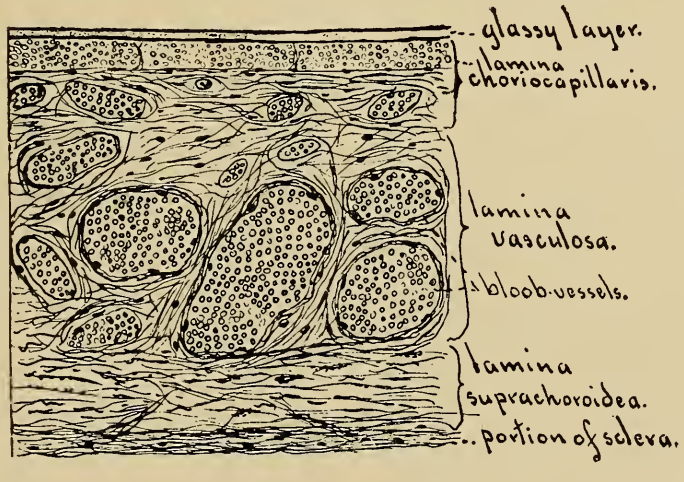


Fig. 65. Human Choroid x 100.

structure? Are pigment-cells present? (4) The vitreous membrane. What is its general structure? Sketch a portion to show these layers and their relative thicknesses and relations. See Fig. 65.

Nervous Tunic. Study the preparation used above. Note that this coat has two layers, (1) the pigment layer and, (2) the retina. What is the shape of the cells in the pigment layer? What is the position of the nuclei? Study the relation of the processes of the cells. The section of the retina lies between the ora serrata and the macula lutea and the following layers should be discerned and carefully studied from without inward: (1) The layer of visual cells which consists of cone-visual cells and rod-visual cells. Try and distinguish them and study their relation carefully. (2) The outer molecular layer. Of what elements does it consist? (3) The inner nuclear layer. What is its structure? (4) The inner molecular layer. What is its formation? (5) The layer of ganglion cells. What type of ganglion cell do you find? Study the cell processes. (6) The nerve fiber layer. Are the fibers medullated or non-medullated? Make a sketch showing these layers and their component parts. See Fig. 66.

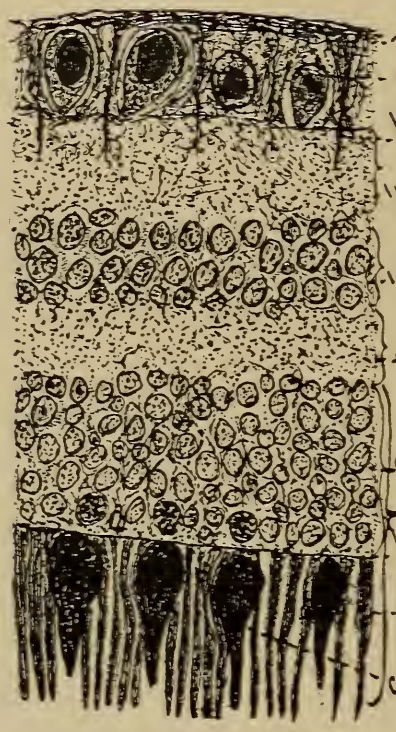


Fig. 66. Retina of Man x 650.

The Crystalline Lens. Macerate the lens for twenty-four hours in Ranvier's one-third alcohol, tease on a slide, cover and study. What is the shape of the fibers? Are they nucleated? How many nuclei? Sketch a few fibers as seen under high power.

The Eyelid. The eyelid was fixed and hardened in alcohol, and imbedded in paraffin. Fix a section to the slide, remove the paraffin, stain in hæmatoxylin and eosin, and mount. Study under low and high power noting the three layers: (1) The cuticular layer with a very thin epidermis. Do you find dermal papillæ? Are they of uniform size and distribution? What variety of glands do you find? Do you find any pigment-cells? (2) The middle layer. What kind of cartilage do you find? What kind of muscle do you find? Note the distribution of blood vessels. What kind of connective-tissue do you find? Has it any definite arrangement? (3) The conjunctiva. What kind of epithelium do you find? Are goblet-cells present? Is lymphoid tissue present? Is it diffuse or nodular? Do you find glands? Sketch a portion of the section to show the structural relation as seen under low power.

The Ear.

External Ear. Portions of the external ear were fixed and hardened in alcohol, imbedded in celloidin, and sectioned. Stain deeply in the elastic tissue stain and mount. Study under low power noting the general features of the section. The skin is rather thin in the external ear. Do you find hair-follicles? Do you find sebaceous glands? Are the sweat-glands present on both surfaces? What kind of cartilage do you find? Are the elastic fibers uniformly distributed? Sketch under low power to show the general structural features.

Inner Ear. The cochlea of a guinea-pig was fixed for twelve hours in Flemming's solution, and after washing, was decalcified in 2% hydrochloric acid (2% nitric acid, or 1% chromic acid would do), imbedded in celloidin, sectioned parallel to the longer axis of the section, and stored in 80% alcohol. Stain in hæmatoxylin and eosin, and mount in balsam.

Study the section carefully under low power. Do you find the axis of the cochlear canal? Do you find the lamina spiralis ossea which separates the canal into the scala vestibuli and the scala tympani? How are these portions of the canal lined? Can you find the cochlear duct? If so try and find the organ of Corti and identify and study its parts. The pillars of Corti form a sort

of arch. Do you find the hair-cells? Look for the lamina reticularis and the membrana tectoria. Try and find the cells of Deiter

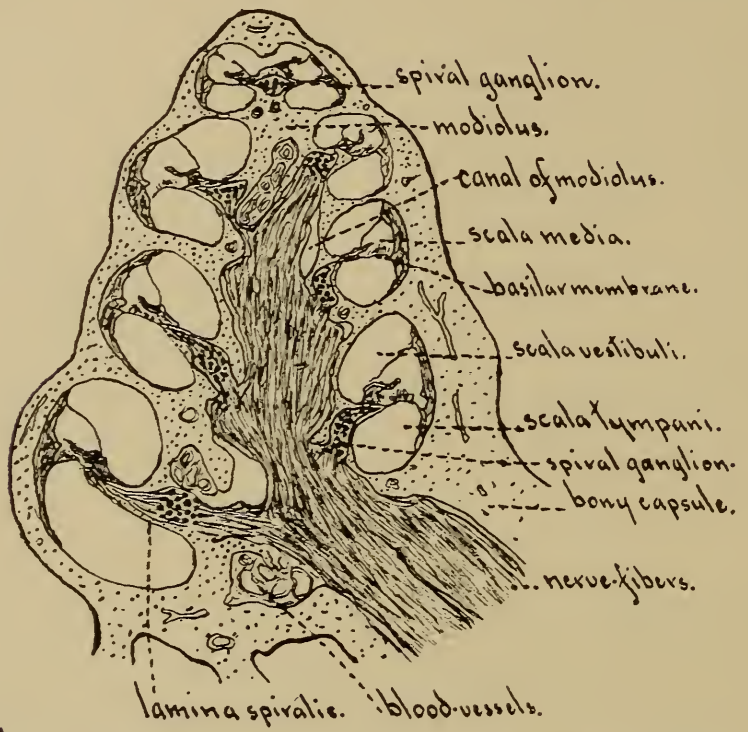


Fig. 67. Long. Section Cochlea of a Guinea-pig. Piersol.

and those of Hensen. Sketch as seen under low power and identify and label all of the parts by comparing with the cuts or with the aid of the instructor. See Fig. 67.

Fœtal Ear. Stain the sections of human fœtal ear in hæmatoxylin and eosin, and mount. Study carefully and compare with the section given above. Sketch and label the important parts.

The Olfactory Mucous Membrane.

Isolated Epithelium. Portions of the mucous membrane of a dog's nose were macerated in Ranvier's one-third alcohol for two hours and were then placed in a 1% solution of osmic acid for twenty minutes. Tease carefully on a slide and examine. Stain another portion with methylene blue, wash, tease, and mount in Farrant's gum glycerin.

Study under high power. What forms of epithelial cells do

you find? How can you distinguish between the olfactory cells and the sustentacular cells? Sketch and label as seen under high power.

Epithelium in Section. Portions of the olfactory mucous membrane were fixed and hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin and eosin, clear, and mount. Study the epithelium, carefully noting its structure. Do you find a basement membrane? Do you find glands? Are they of the serous or mucous type? How are the blood vessels distributed? Sketch to show the most important structures. See Fig 68.

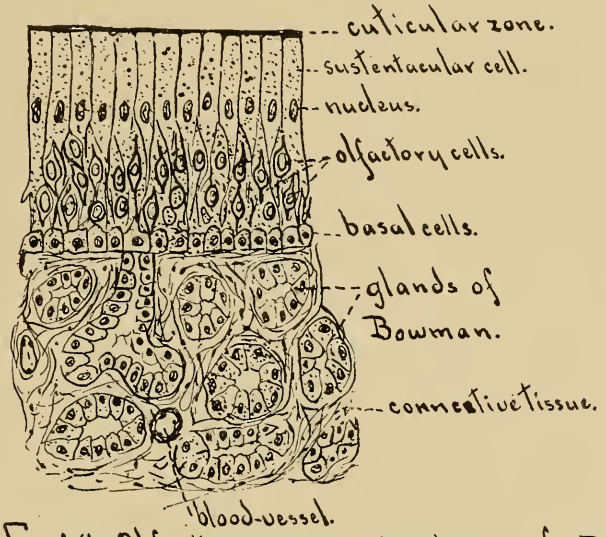


Fig. 68. Olfactory Mucous Membrane of a Boy.

Nerve Histology.

Nerve Cells. With a scalpel remove a small portion of the gray matter from the anterior horn of the spinal cord of the ox, place it between two clean cover-glasses and make a cover-glass preparation or smear. Allow the smear to dry in the air and then place it in cover-glass for-

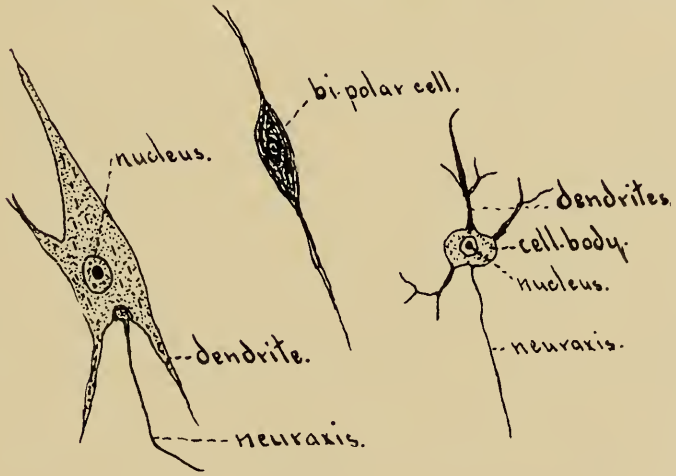


Fig. 69. Nerve-cells Spinal Cord of Ox.

ceps and pass quickly two or three times through the flame of a Bunsen burner. Stain for twenty or thirty minutes in a 1% aqueous solution of methylene blue. Wash in water, dry thoroughly between pieces of filter paper, or in the air, and mount in balsam.

Study the preparation under low and then under high power. Note the general form of the cell-body. Are the cells unipolar, bipolar, or multipolar? Do you find a neuraxis? Are the cells alike as to form and size? Is the protoplasm clear or granular? Can you see any fibrillation in the protoplasm under the high power? Are nucleoli present? Can you find the implantation zone? Make drawings. See Fig. 69.

Nerve Cells Macerated. Macerate portions of the gray matter from the anterior horns of the spinal cord for a week or ten days in ⅓ alcohol, a 1% solution of potassium dichromate, or a 5% solution of chromic acid. Thionin is a good stain to use after alcohol as a fixative. Lithium carmine after chromic acid is recommended by Huber. The stained material should be carefully teased and examined in glycerin, or it may be mounted in gum glycerin. Study and note the structure and form as above. You should be able to trace portions of the neuraxes. Sketch a cell showing the neuraxis.

Nerve Cells of the Cerebral Cortex. Small pieces of the cerebral cortex of a cat were placed in the following mixture, as recommended by Fish:

Formalin 2 c. c.
Potassium dichromate, 3%, 100 c. c.

for three days, using fifty times their volume of fluid, and kept in the dark. The tissue was then placed in the 3% potassium dichromate solution for three or four days. The potassium dichromate was replaced by a ½% solution of silver nitrate until the chromate of silver was no longer precipitated. A fresh solution of silver nitrate was poured over the tissue and it was kept in the dark for from three to five days. It is best to change the silver nitrate solution after ten to twelve hours. The tissue was then imbedded in celloidin as follows: Dehydrate in 95% alcohol, 15-20 minutes. Change the alcohol once or twice and agitate. Absolute alcohol for 30-40 minutes. Ether-alcohol, 20 minutes. Ether, 15 minutes. Thin celloidin, 30 minutes. Thick celloidin, 15 minutes. Tissue was mounted on blocks and placed in chloroform for 10 minutes,

after which it was placed in a modified Eycleshymer's clearing fluid (carbolic acid, 25 parts; oil of cedar, 50 parts; and oil of bergamot, 50 parts), for one hour. The tissue may remain in oil a day or two without seeming to suffer much injury. Sections were cut with a sliding microtome, using the clearing fluid to wet the tissue and the knife, and transferred to a dish of clearing oil. Take a section from the clearing oil and place it on a clean slide, press down and blot with a piece of filter paper, and cover with a large drop of *very thick* Canada balsam. *Do not use a cover-glass* and dry quickly in a warm, dry place protected from dust.

Study the section with low power. The plexus of blood vessels may confuse you but search for pyramidal cells, stained a brownish-black, with a single neuraxis extending from the base of the cell-body into the white matter, and branching dendritic processes extending toward the surface of the cortex. Only portions can be found in connection with a single cell. Note the size of the dendrites as compared with the neuraxis. Do the cells have a definite arrangement? Could they be said to have a characteristic form? Do they have more than one dendrite? Note the branching of the dendrite. Sketch several pyramidal cells and label the parts. See Fig. 70.

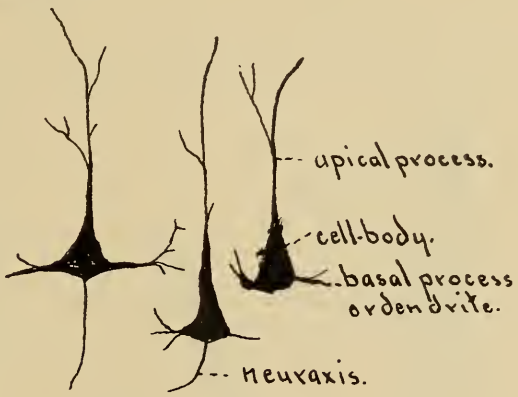


Fig. 70. Pyramidal Cells of a Cat. Golgi.

Nerves.

Fresh Fibers. Carefully remove the sciatic nerve from a frog and place it in physiological normal salt solution (.6%). With very sharp scissors remove a piece about one-half inch in length and place it on a slide and tease carefully keeping the fibers as nearly straight as possible; cover with a cover-glass and examine first with low power, and then with the high power. Use a small diaphragm opening for the high power.

Search for the neuraxis or axis cylinder seen as a light band running down through the center of the fiber. Outside of the

neuraxis is the myelin sheath which has a glistening appearance with a slightly greenish tint. Do you find the nodes of Ranvier? Estimate the length of the internodal segments. Look for the neurilemma as it crosses from one internode to another. Is the medullary sheath continuous from internode to internode? Do you find the nuclei of the internodal segments? Look for the segments of Lantermann. Do you find the long, oval neurilemma-nuclei? Note the contraction ring of the neurilemma at the nodes. Are all the fibers of uniform diameter? Do they branch? Make a sketch to show as many of these points as possible.

Stained Nerve Fibers. The sciatic nerve of a frog, or a small nerve of a mammal, was placed in a .5% solution of osmic acid for twenty-four hours, washed in water, and stored in 70% alcohol. Take the portion given you and tease carefully on a slide in dilute glycerin, cover and examine, first with the low and then with high power. The medullary sheath is stained a deep black. Why? Can you trace the neuraxis? Study the nodes and the neurilemma contraction ring. Do you find the segments of Lantermann? Sketch a node of Ranvier with portions of the internodal segments as seen under high power. See Fig. 71.

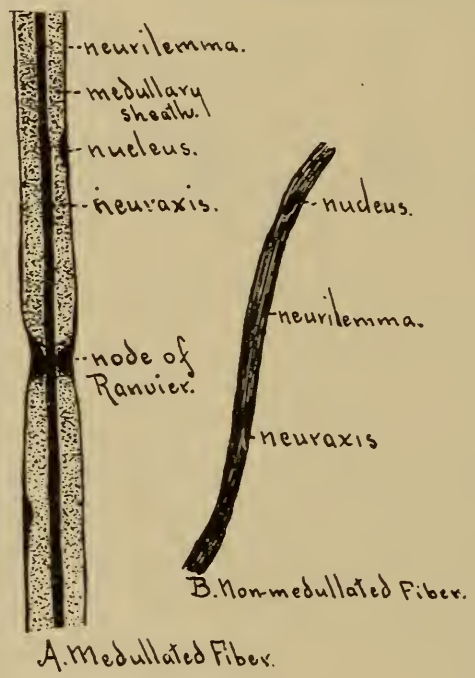


Fig. 71. Nerve Fibers.

Stained Nerve. A section of human ulnar nerve was hardened in formalin, imbedded in paraffin, and sectioned longitudinally. Fasten to a slide with albumin fixative and stain in hæmatoxylin, Boehmer's is best. Study under high power. What is the appearance of the medullary sheath? Are the internodal nuclei visible? The neuraxis usually stains deeply. Sketch a portion of the section.

Stained Nerve. A cross section of the same nerve was hardened in Müller's fluid, inbedded in celloidin, and sectioned. Stain

in aniline blue and safranin, dehydrate, clear in oil of bergamot, and mount in balsam. Study under low power noting the epineurium

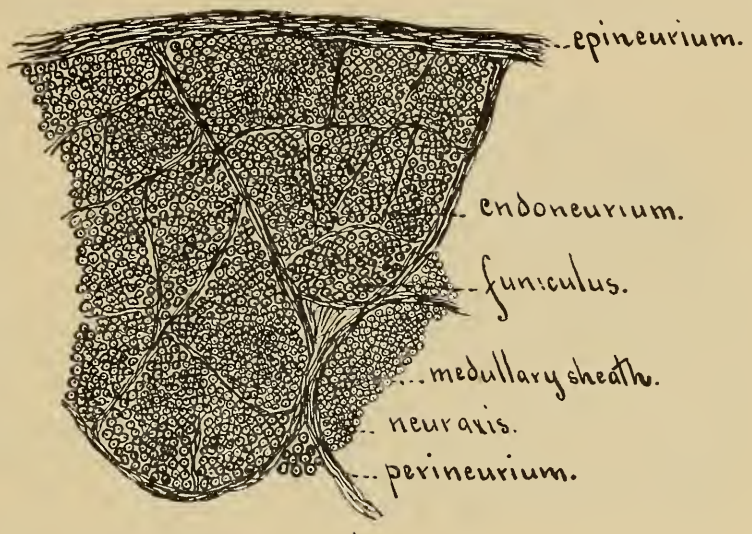


Fig. 72. T.S. Human Ulnar Nerve.

and the perineurium. The neuraxes are stained blue, the nuclei red, and medullary sheaths a reddish-yellow. Sketch a portion carefully, labeling all of the parts. See Fig. 72.

Non-medullated Nerves. (a) The splanchnic nerves of a dog were fixed in formalin, and stained in hæmatoxylin. Tease carefully in glycerin and study under the high power. Are the fibers medullated or non-medullated? Do the non-medullated fibers possess nodes of Ranvier? Do they have a primitive sheath or neurilemma? Do you find nuclei? Sketch a typical non-medullated fiber. See Fig. 71.

(b) Pieces of the vagus nerve were treated with a .5-1% solution of osmic acid for twenty four hours and they are to be carefully teased and examined in dilute glycerin. Between the medullated fibers the non-medullated are found. Compare with (a) above and sketch a few fibers.

Ganglia. Cross sections of the spinal cord of a cat were made so as to pass through the posterior root ganglia. Stain deeply in Ehrlich's acid-hæmatoxylin and Van Gieson's stain. Dehydrate, clear in Eycleshymer's nerve mixture, and mount in balsam. Study under low power. Note the capsule of the ganglion and its septa

and trabeculæ. How are the ganglion cells arranged? Study the cells under high power. Are they unipolar or bipolar? Note the large nucleus and the nucleolus. What is the relation of the

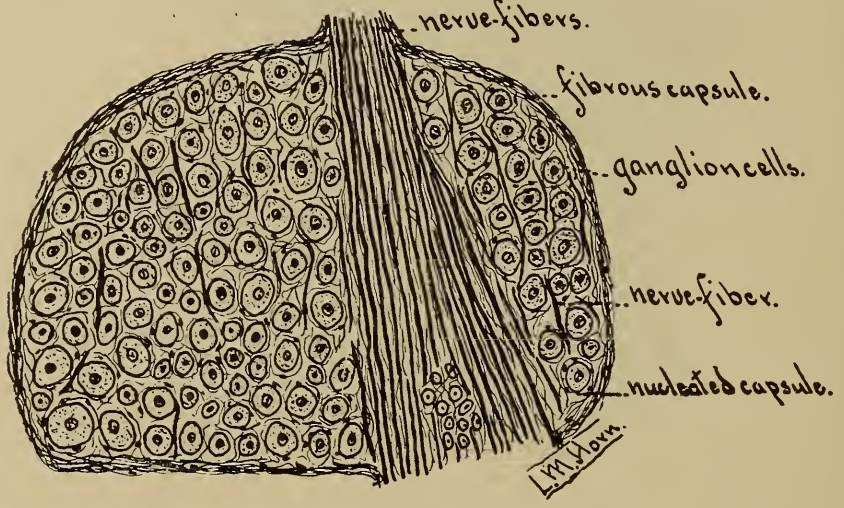


Fig. 73. Section of Cervical Ganglion of Cat x 90.

cells to each other? Do you find any fibers? Do any fibers end in the ganglion? Sketch under low power to show general relation and arrangement, and under the high power to show as much as you can of the ganglion cell and its structure. See Fig. 73.

Nerve Endings. Treat pieces of the ocular or intercostal muscles, other small muscles would do as well, by Ranvier's lemon-juice method as follows: The lemon-juice should be filtered through clean new flannel, after which pieces of the tissue are placed in it until they become transparent, usually requiring five or ten minutes, after which they are to be quickly rinsed in distilled water and placed in the dark in a 1% solution of gold chloride for twenty or twenty-five minutes, after which they are to be washed in water and placed in distilled water to

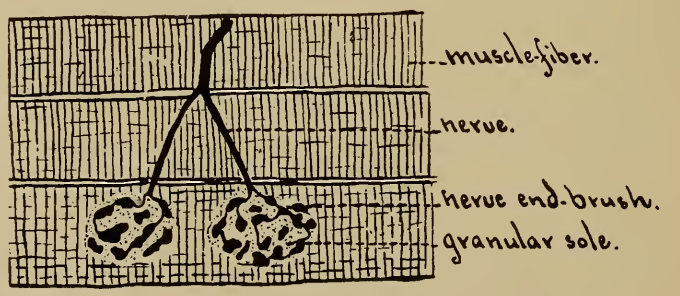


Fig. 74. Motor Nerve-ending. Striated Muscle.

which acetic acid has been added in the proportion of one drop of acid to each 25 c. c. of water. Now expose to the light for at least twenty-four hours. The reduction of gold is not very complete and such material is only suited for present study. Permanent preparations may be made by placing the tissue in formic acid of 25% strength. The reduction is usually complete in twenty-four hours.

Tease the tissue very carefully on a slide and search for the motor-endings. Study carefully and make several sketches to show typical forms of endings. See Fig. 74.

Crosses of Ranvier.—Silver Nitrate Method. Place portions of a small nerve, or spinal nerve roots, in a 1% solution of silver nitrate. Keep it in the dark for about twenty-four hours. Remove from the silver solution, rinse quickly in distilled water and place on a slide in a drop of glycerin and carefully tease one end of the fiber, cover, and expose to the sunlight for twenty-five to thirty minutes.

Study under high power. Do you find the crosses? Why are crosses formed at the nodes? Do you find the transverse striations known as Frommann's lines? Sketch.

Spinal Cord.

A portion of spinal cord from the cervical region of a cat was hardened in corrosive sublimate in normal salt solution, or in formalin. After further treatment with iodized alcohol and pure alcohol, in case the sublimate be used, the tissue was imbedded in celloidin, and sectioned. You will stain the section on a slide in borax carmine, dehydrate, clear in Eycleshymer's mixture, and mount in balsam.

Study under the low power and note the form of the cord, fissures, membranes and nerve-roots. Examine the gray matter, noting the relative size and position of the anterior and posterior cornu. Do you find any definite regions or cell areas in the gray matter? Name them by consulting the figure. Examine the white matter more carefully, trying to see if it is divided into definite areas. Sketch the cross-section under the low power being careful to preserve the proper proportions and to keep the relative size of the various parts. See Fig. 75.

A section of the spinal cord of an ox was hardened in fluid consisting of 3% potassium bichromate, 98 parts, and formalin, 2 parts. The tissue was imbedded in celloidin and sectioned. Stain the section you receive in Weigert's hæmatoxylin, wash, dehydrate,

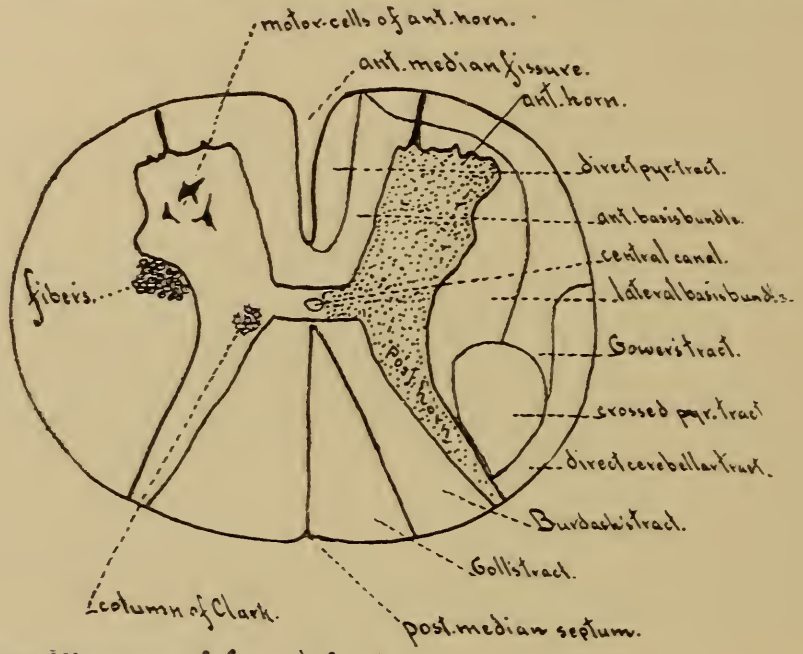


Fig. 75. Diagram of Spinal Cord.

clear, a xylol-carbolic acid mixture is good, or in Eycleshymer's nerve fluid, mount in balsam, and study as above, noting the finer details in the structure under the high power. Do not attempt to make a complete sketch but fill in portions of both gray and white matter in a complete outline sketch of the cord.

This portion was stained by the rapid Golgi method (mentioned on page 72), imbedded in celloidin, and sectioned. Remove the excess of clearing oil and mount in *very thick* balsam, *without a cover-glass.* Study under low power and make drawings of typical cells and structures.

Spinal Cord Stained in Nigrosin and Eosin. Stain the section for one hour in a 1% aqueous solution of nigrosin. Wash in distilled water for five minutes. Stain for five minutes in a 1% solution of eosin. Wash in 70% alcohol for several minutes and place in 95% alcohol until stain ceases to be given off. Clear in oil of bergamot for ten to fifteen minutes, and mount in balsam. Study as above.

cells do you find, large or small ganglion cells? What tissue seems to form the greater part of this layer? (2) Cell layer of Purkinje's cells. Can you trace an axon from the base of the cell-body? How many dendritic processes at the opposite pole? How do they branch? Do the branches seem to have varicose enlargements? Do they anastomose? Do you find basket cells? (3) Granular layer containing small granular (ganglion) cells and large stellate cells. How many dendrites do the small cells have and how do they end? Can you trace the neuraxes toward and into the molecular layer? Look for the large stellate cells close to the molecular layer, noting the branching and course of the dendrites and neuraxes. Sketch a portion showing as much as you can of the cortical layers. See Fig. 76.

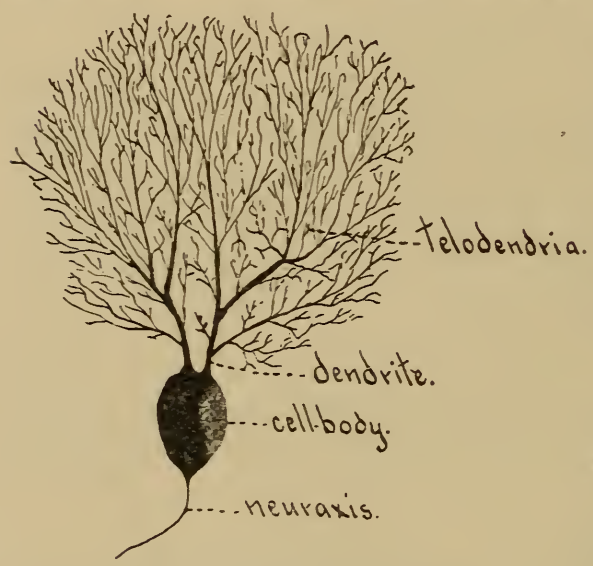


Fig. 77. Cell of Purkinje of a Cat.

Golgi Method. Portions of the cerebellar cortex were treated by the rapid Golgi method mentioned on page 72. Mount as there described and study and compare with the above. Sketch as seen under low power, and sketch several cells of Purkinje as seen under high power. See Fig. 77.

The Cerebrum.

Stained Cortex. Small pieces of the cerebral cortex of a cat were fixed in bichloride of mercury, hardened in alcohol, imbedded in celloidin, and sectioned. Stain in hæmatoxylin, and, very lightly, in eosin, dehydrate, clear, and mount in balsam. This section is useful for the purpose of showing the general layers. What kind of tissue composes the larger portion of the molecular layer? Do you find any nerve cells? Look for a thin stratum of small medullated fibers just beneath the pia mater. Is the molecular layer sharply marked off from the layer beneath it? Describe the

structure of the granular layer. Note the relative number of cells in the granular and molecular layers.

Golgi Cortex. Pieces of the cerebral cortex were prepared by the rapid Golgi method described on page 72, imbedded in celloidin, and sectioned. Remove the excess of clearing fluid and mount in a drop of *very thick* balsam, *without using a cover-glass*. Study under low power, noting carefully the form and relative number of cells in each layer. How are the cells distributed in the outer layer? Do they possess typical forms? Do you find distinct boundaries between the layers? Note the relative size of the cells of the pyramidal layers. From what portion of the cell body is the axon given off? How many dendrites, and where do they terminate? What forms of cells do you find in the layer beneath the layer of large pyramidal cells? Make a sketch showing the structure of the cortex. See Fig. 78.

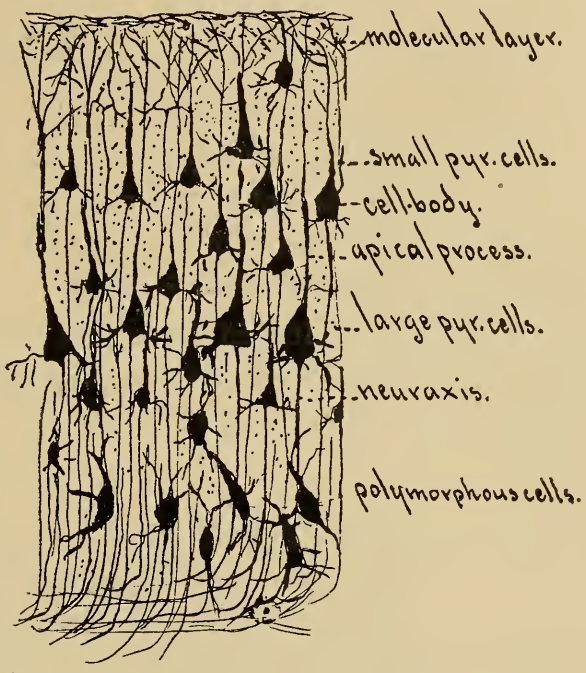


Fig. 78. Cortex of Brain. Golgi Method.

Nerve Fibers of the Cortex. A portion of the cerebral cortex of a dog was fixed in Müller's fluid, hardened in alcohol, imbedded in celloidin and sectioned. Stain the sections in Weigert's hæmatoxylin, dehydrate, clear, carbol-xylol is best for clearing, and mount. The nerve fibers are stained a blue-black color. Note carefully the distribution of nerve-fibers in the cortex. Sketch a portion of the cortex, using the low power.

Formulæ for Reagents.

A. STAINS.

(1) Bœhmer's Hæmatoxylin.

Hæmatoxylin crystals, 1 gram.
Absolute alcohol, 10 c. c.
Potassium alum, 10 grams.
Distilled water, 200 c. c.

Dissolve the alum in the water and the hæmatoxylin in the alcohol. Mix while stirring constantly. Place in an open jar, protect from dust, and let ripen for two or three weeks. Filter, and the stain is ready to use.

(2) Delafield's Hæmatoxylin.

Hæmatoxylin crystals, 4 grams.
Absolute alcohol, 25 c. c.
Sat. aq. solution of ammonia alum, 400 c. c.
95% alcohol, 100 c. c.
Glycerin, 100 c. c.

Dissolve the hæmatoxylin in the absolute alcohol and add to the solution of alum. Place in an open dish and expose to the light for four or five days and filter. Add the 95% alcohol and the glycerin and let the solution ripen for six or eight weeks. It is now ready for use after filtering. It is diluted with distilled water before using.

(3) Ehrlich's Acid Hæmatoxylin.

Hæmatoxylin crystals, 2 grams.
Absolute alcohol, 100 c. c.
Glacial acetic acid, 10 c. c.
Glycerin, 100 c. c.
Distilled water, 100 c. c.
Potassium alum, in excess.

This stain should ripen in the light for some time until it acquires a dark-red color. It stains and keeps well for years if kept in well-stoppered bottles.

(4) Aqueous Solution of Borax–Carmine.

Grind 2 grams of carmine with 8 grams of borax in a mortar, and add it to 150 c. c. of distilled water. Let it stand for from twenty-four to thirty-six hours and filter when it is ready for use.

(5) Safranin.

Safranin, 1 gram.
Aniline water, 90 c. c.
Absolute alcohol, 10 c. c.

Prepare the aniline oil by shaking 8 to 10 c. c. of aniline oil in 100 c. c. of distilled water, and filtering through a wet filter. Dissolve the safranin in the aniline water and then add the alcohol. It must be filtered before using.

(6) Weigert's Hæmatoxylin.

Hæmatoxylin crystals, 1 gram.
Absolute alcohol, 10 c.c.
Lithium carbonate, 1.2 grams.
Distilled water, 100 c. c.

Dissolve the hæmatoxylin crystals in alcohol, the lithium carbonate in water, and mix the two solutions.

(7) Congo Red.

Make a .5-1% aqueous solution. It is particularly useful in staining the parietal cells of the cardiac end of the stomach since it seems to have a special affinity for acid. It is also used as a stain for the axis-cylinder of a nerve fiber.

(8) Thionin.

A concentrated aqueous solution is usually used. It stains chromatin readily, and stains plasmatic elements if the staining process be prolonged. It is more used as a specific mucin stain.

(9) Orcein, Israel's Formula.

Orcein, 2 grams.
Glacial acetic acid, 2 c. c.
Distilled water, 100 c. c.

This gives a blue stain in the nucleus and a red stain in the cytoplasm. After staining wash the section in distilled water, dehydrate rapidly, passing through absolute alcohol to *thick* cedar oil in which it is mounted.

(10) Van Gieson's Stain.

(a) Make a saturated aqueous solution of acid fuchsin.
(b) Make a saturated aqueous solution of picric acid.
Add (b) to (a) until the mixture is garnet red.

(11) Elastic Tissue Stain of H. G. Harris.

Hæmatoxylin, 0.2 gram.
Aluminium chloride, 0.1 gram.
50% alcohol, 100 c. c.

(12) Acid Fuchsin.

Use a 0.5-1% aqueous solution.

(13) The Ehrlich-Biondi Mixture.

Take 200 c.c. of a saturated aqueous solution of orange G and add to it while stirring 40 c.c. of a saturated aqueous solution of acid fuchsin, and 100 c.c. of a saturated aqueous solution of methyl green. The saturated solutions should be prepared several days before using and they must be completely saturated. Sections should be stained for from twelve to twenty-four hours, dehydrated, cleared with xylol, and mounted in xylol-balsam.

(14) Heidenhain's Iron-Hæmatoxylin Stain.

Hæmatoxylin crystals, 1 gram.
Absolute alcohol, 10 c. c.
Distilled water, 90 c.c.

To be diluted with an equal volume of distilled water when used. A mordant of a 2.5% solution of ammonium sulphate of iron is used for five or six hours. After staining for twenty-four hours, the ammonium sulphate of iron solution is used to remove the excess of stain.

(15) Eosin.

Eosin is commonly used as a 2% solution in 60% alcohol, though saturated aqueous solutions may be used as well as saturated alcoholic solutions.

B. MACERATING FLUIDS.

(1) Ranvier's One-third Alcohol.

Fresh tissue should be macerated from eighteen to twenty-four hours, after which it may be teased on a slide in a drop of dilute

glycerin. Or, after macerating, treat for several hours in a 5% solution of osmic acid before teasing.

(2) Hydrochloric Acid.

Hydrochloric acid in a 25-30% solution is commonly used to macerate kidney tissue. It should act at least twelve hours after which the tissue is washed and teased in water or dilute glycerin.

(3) Caustic Potash.

A 30% aqueous solution is much used for macerating cardiac and smooth muscle. It should act for about twenty minutes after which it is placed in a saturated aqueous solution of potassium acetate to which a little glacial acetic acid has been added in the proportion of about one drop of acid to 5 c.c. of the acetate solution. After twenty-five minutes the tissue may be teased and examined.

(4) Nitric Acid.

It may be used in a 30% solution, or in a 20% solution of fuming nitric acid.

(5) Chromic Acid.

This is used in aqueous solutions of from 0.2-1% strength. It is used to macerate smooth muscle, and may be used for nerve tissues.

C. FIXING FLUIDS.

(1) Bichloride of Mercury.

A saturated solution in distilled water is used. A saturated solution in physiological normal salt solution (.6%) makes a good fixing agent.

Huber combines it with formalin. This seems to be useful in embryonic tissue, especially developing bone. He adds four parts of formalin to one hundred parts of a saturated aqueous solution of the bichloride of mercury.

(2) Müller's Fluid.

Bichromate of potassium, 2.5 grams.
Sulphate of sodium, 1 gram.
Distilled water, 100 c.c.

This solution is much used for nerve tissues and for large pieces of tissue. One objection to its use is that it acts *very slowly*, and

hence it should not be used if the finer details of cell structure are desired, as post mortem changes usually occur to some extent before the killing and fixing process begins.

(3) **Flemming's Solution.**

2% aqueous solution of osmic acid, 4 c.c.
1% aqueous solution of chromic acid, 15 c.c.
Glacial acetic acid, 1 c.c.

It is best to keep the osmic acid and chromic acid in separate bottles and combine them and add the acetic acid when wanted for use.

(4) **Carnoy's Fluid.**

2 or 3% aqueous solution of chromic acid, 45 c.c.
2% aqueous solution of osmic acid, 16 c.c.
Glacial acetic acid, 3 c.c.

(5) **Alcohol.**

Small pieces of tissue may be placed in absolute alcohol, being killed, fixed, hardened, and dehydrated at the same time. Unless absolute alcohol is used, 35% alcohol should be used for a few hours, followed by 50%, 70%, 85%, etc., as tissue placed directly into 95% alcohol suffers considerable shrinkage.

(6) **Formalin.**

Formalin in a 5-10% solution is much used, especially for nerve tissues. It is not best to use it in case white fibrous connective tissue predominates in the section as it hardens it very greatly. Nor is it a good fixing agent for portions of such tissues as the stomach as it does not harden the tissue uniformly and it does not cut well.

(7) **Chrom-acetic Fluid.**

Chromic acid, 2 grams.
Glacial acetic acid, 2 c.c.
Water, 296 c.c.

Mayer's Albumin Fixative.

This is made by taking equal parts of white of egg and glycerin and adding to it a little salicylate of soda or gum camphor to prevent decomposition.

Acid Alcohol.

Add to 50% or 60% alcohol about 2% of hydrochloric acid.

Ammonia Alum.

For use in removing the excess of hæmatoxylin after over-staining take a 2-3% aqueous solution of ammonia alum.

Lugol's Solution.

Distilled water, 100 c.c.
Potassium iodide, 6 grams.
Iodine, 4 grams.

Carmine-gelatin Injection Mass. (Ranvier.)

Soak fifty grams of Paris gelatin in water for thirty minutes, or until it becomes soft, and then melt it in the water it has absorbed in a porcelain vessel over a water-bath. When it is completely melted add a solution of five grams of carmine in just enough ammonium hydrate to effect the solution of the carmine forming a transparent solution. Stir the mixture constantly keeping it warm over the water-bath. The *excess* of ammonia must be neutralized. Add drop by drop, while stirring, a solution of one part of glacial acetic acid to three parts water. When you are near the point of neutrality dilute the acid still further. The smell gradually changes from ammoniacal to the sour odor of the acid. This must be done slowly and carefully as a single drop of acid in excess may spoil the mixture. Filter through clean flannel.

Farrant's Gum-glycerin.

Glycerin, 50 c.c.
Distilled water, 50 c.c.
Pure gum-arabic, 50 grams.
Arsenious acid, 1 gram.

Dissolve the acid in water. Mix with the gum-arabic in a mortar. Add the glycerin and filter through fine new muslin.

Stock or Thick Solution of Celloidin.

Dissolve 12 grams Schering's celloidin in 200 c.c. of equal parts of absolute alcohol and ether. A thin solution is prepared by taking equal parts of the stock solution and the absolute alcohol and ether solution.

D. CLEARING FLUIDS.

Purposes of a clearing oil. The oil removes the alcohol, renders the section more transparent, and is a solvent for the mounting media.

(1) Oil of Cloves.

Clears quickly, does not spread over the slide, and renders the sections somewhat brittle. Dissolves celloidin. Clears from 95% alcohol.

(2) Oil of Bergamot.

Clears quickly from 95% alcohol. Does not destroy aniline stains. Does not dissolve celloidin.

(3) Oil of Origanum.

Does not dissolve celloidin if good. Clears from 95% alcohol.

(4) Carbolic Acid.

Clears quickly and does not dissolve celloidin.

(5) Eycleshymer's Mixture.

Oil of cedar, one part.
Oil of bergamot, one part.
Carbolic acid, one part.

Does not dissolve celloidin, clears rapidly from 95% alcohol, and is a splendid clearing agent.

(6) Eycleshymer's Nerve Mixture.

Oil of cedar, 50 c.c.
Oil bergamot, 50 c.c.
Carbolic acid, 25 c.c.

(7) Xylol–Carbolic Acid Mixture.

Xylol, three parts.
Carbolic acid crystals, one part.

(8) Xylol.

Clears readily after chloroform or absolute alcohol, but not after 95% alcohol. Used on paraffin sections only.

(9) Ether–Alcohol.

Take equal parts of ether and of absolute alcohol.

INDEX